STUDIES IN ANTHELMINTICS
WITH SPECIAL REFERENCE TO
IN VITRO ANTHELMINTIC ACTIVITY
AND CHEMICAL CONSTITUTION

By

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PART I.

The prevalence of helminthiasis is so serious, not only amongst the human population of the world but also amongst our domestic animals, that it is surprising that more research, especially of a fundamental nature, has not been carried out. The annual loss in farm animals due to helminthic infestations adds to the difficulty of solving the world's food problem (cf. Lapage, 1948).

Many substances with anthelmintic properties are known and a number are employed in medical and veterinary practice. The earliest anthelmintics used were obtained from plants, and it is only within comparatively recent times that synthetic organic compounds have been employed as such.

There has been a relatively small amount of systematic work done on anthelmintics. Several possible causes for this present themselves, e.g., insufficient collaboration between zoologist and chemist, the very numerous species of helminths and their somewhat complex life histories, the fact that they cannot be cultured *in vitro*, the difficulty encountered in devising suitable *in vitro* and *in vivo* methods of testing, and insufficient information regarding helminth physiology.

Research/

Research in anthelmintics may be approached from different angles. A study may be made, for example, of the effect of known anthelmintics on the host as well as on the parasite, and of the metabolic changes or the fate of the anthelmintic in the host. In some cases, the metabolite probably is the active anthelmintic. Compounds may be prepared which have also no direct anthelmintic action, but which interfere with the normal metabolism of the parasite, and thereby reduce its activity, with consequent mechanical removal from the host, or produce a lethal effect.

The search for new and more efficient anthelmintics is necessary and the development of satisfactory screening tests is essential, so that a large variety of chemical compounds may be examined expeditiously.

The solution of the problem of combating helminthiasis may be facilitated by finding chemicals which are lethal to the larval or other pre-adult forms of the helminths and to intermediate hosts.

Chemical Constitution and Anthelmintic Effect. - Systematic studies of a fundamental nature would now appear to be necessary before any real advance can be made in the field of anthelmintics. This is more or less indicated by Bueding (1949). "Routine testing programs/

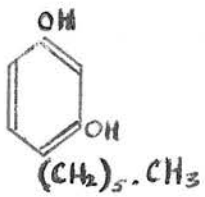
programs, in which large numbers of unrelated compounds have been assayed for anthelmintic activity, have constituted the principal approach to the experimental chemotherapy of helminthic infestations up to the present time. In the course of such empirical studies, little knowledge has been gained concerning the mechanism of action of existing anthelmintic agents and the basic physiology of the parasites involved, despite the great potential value that such information would have in suggesting new and more logical approaches to the problem."

This thesis describes such a fundamental approach, and presents a study of the relationship between chemical constitution and in vitro anthelmintic effect. Previous investigations of a related character led to conflicting results. Baldwin (1948) attributed this to the employment of unsuitable methods of in vitro testing, and so far comparatively few definite results are available. In the homologous series of organic compounds some correlation has been indicated. Wright and Schaffer (1932) carried out investigations on a number of chlorinated hydrocarbons and found that chemical composition did have an effect on anthelmintic potency, although solubility in water was an important factor. 2-Chloropentane (I) was at least four times more efficient than 2-chlorobutane (II) in the removal of hookworms (Ancylostoma caninum) from the dog.

$CH_3.CHCl.CH_2.CH_2.CH_3$ (I) $CH_3.CHCl.CH_2.CH_3$ (II)

Lamson/

Lamson et al. (1935, 1936) carried out a systematic investigation of the anthelmintic properties of the alkylhydroxybenzenes and found that, in the case of the alkyl mono- and dihydric phenols, ascaricidal activity increased with increase in the length of the carbon chain of the substituent, but a maximum was reached, which differed in different series, and a rapid fall in activity was observed in the higher members. 4-n-Hexylresorcinol (III) was the most active of the 4-n-alkylresorcinols. It was also found that certain chlorinated alkyl phenols had marked in vitro



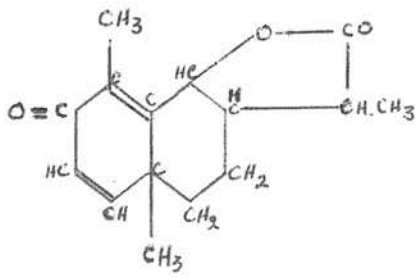
(III)

ascaricidal properties.

More recently Williams, Schelling, and Hartmann (1949) studied the effect of a number of alkylhydroxybenzenes on Ascaris lumbricoides in vitro and also showed that halogens, as substituents, increased the anthelmintic potency of a compound. 2-Ethyl-6-chloro-4-n-hexylresorcinol appeared to be more active than 4-n-hexylresorcinol in vitro. The ethyl group may have/

have exerted an influence on the activity but, in all probability, the chlorine was the more potent substituent.

Moreover, certain structures appear to be associated with anthelmintic activity. It has been suggested, for example, that the activity of the anthelmintic santonin (IV), obtained from various



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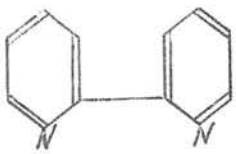
species of Artemisia, is due to its lactone structure (Trendelenburg, 1915), and other lactones have been tested and claimed to possess 'anthelmintic' properties (Lautenschläger, 1921; von Oettingen, 1929; Gluschke, 1932; Rosenmund and Schapiro, 1934; Baldwin, 1948). The presence of the ketonic group in santonin may also be a contributary factor, as Baldwin (1948) has shown that aliphatic-aromatic and aromatic-aromatic ketones produced anthelmintic effects, although none of the ketones possessed the potency of santonin. (see also Caius and Mhaskar, 1923). Baldwin (1948) suggested that the outstanding activity of santonin may be due, not/

not to the presence of a keto-group and a lactonic structure alone, but to their relative position to each other in the molecule. Moreover, he (1943) had found that pilocarpine, strophanthin, and umbelliferone, all of which possess a lactonic structure, had no anthelmintic effect on A. lumbricoides in vitro.

Baldwin (1948) tested over 200 chemical compounds, ketones, lactones, phenols, thiazoles, pyridines, and miscellaneous antibiotics, in vitro against A. lumbricoides and showed that certain groups did affect anthelmintic potency, and that the influence was modified by the position of the substituent groups in the molecule. The ketonic group appears to induce anthelmintic activity which can be enhanced by the presence of hydroxy or alkyloxy groups in the benzene nucleus or by halogenation. Baldwin also found that some of the lactones tested showed definite activity, but points out that from his observations this activity may be due to the presence of other groups, etc. in the molecule. It was found, however, that the compounds with separated rings were more potent than those with a condensed ring system. This latter observation was also made when the phenols were tested, which also showed considerable activity. The thiazoles were not promising, but amongst the pyridines were compounds of high activity, particularly those with separated/

separated rings. A compound of considerable potency was 2:2'-dipyridyl (V), but the activity was much reduced in the other isomeric dipyridyls. Similarly 4:5-phenanthroline (VI) was much more active than the



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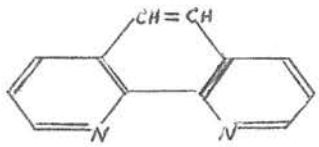


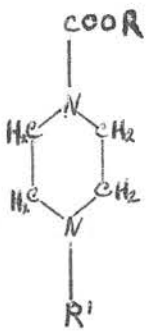
(VI)

other phenanthrolines tested, and Baldwin came to the conclusion that the high activity was due to the presence in these two compounds of the bond system.

The miscellaneous antibiotics were found to have no anthelmintic action.

Hewitt et al. (1947, 1948) carried out investigations on filariasis using piperazine derivatives. In these studies also, it was found that the antifilarial potency was influenced by the substituents in the piperazine/

piperazine nucleus. In the series of compounds (VII), for example, it was found that the carbethoxyl group, ($R = C_2H_5$) appeared to be more effective than when $R = CH_3$, $\underline{n}\text{-}C_4H_9$, $\underline{iso}\text{-}C_4H_9$, and, when R was kept the same and R' varied, the most potent compound was

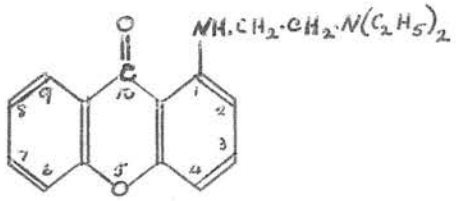


(VII)

found to be when $R = C_2H_5$ and $R' = CH_3$.

A number of basic-substituted xanthones and thioxanthones have been prepared by Mauss, some of which showed significant activity in the treatment of schistosomiasis in experimental animals (B.I.O.S. Final Report, No. 116, Item No. 24; C.I.O.S. Report, Item No. 24, File No. XXV-54; Mauss, 1948; Kikuth, Gönnert, and Mauss, 1946). It was found that certain variations in composition produced differences in activity. 1-2'-Diethylaminoethylamino-xanthone (VIII),/

(VIII), for example, showed no activity against



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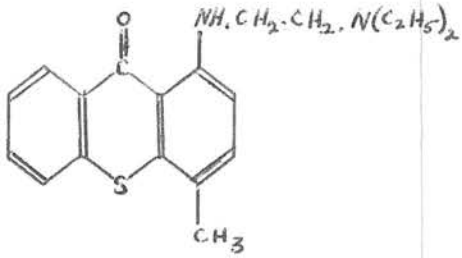


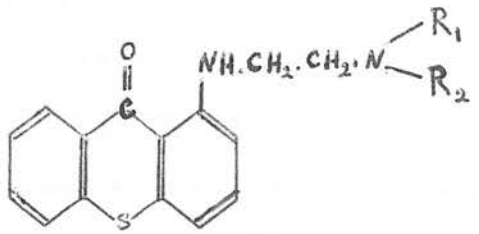
(IX) ⁂

schistosomes, whereas 1-2'-diethylaminoethylamino-4-methyl-xanthone (Miracil A) was active. It was demonstrated that a methyl group in the p-position to the basic group was essential for activity. Replacement of this methyl group by ethyl, or methoxyl groups, or a chlorine atom led to loss of activity. In the xanthone series decrease in activity was generally observed when the number of carbon atoms in the side chain exceeded two. Derivatives of Miracil A with the substituents methyl, methoxy, or chlorine in the 7- or 8-position were prepared. The 7-chloro-derivative (Miracil B) was the most active. In the thioxanthone series, similar results were obtained. The most effective compound was 1-2'-diethylaminoethylamino-4-methylthioxanthone (Miracil D, Lucanthone, B.P.) (IX).

⁂ The American system of numbering is adopted for these and other 3-ringed compounds, since there appears to be some inconsistency in the British system (cf. Mitchell, 1948).

Levine (1951) and Levine and Ivens (1953) tested ninety-four inorganic and organic iodine compounds against horse strongyle larvae and/or eggs in faeces. They found that inorganic unionised compounds, aryl iodides, and polyiodo-compounds are relatively inactive compared with inorganic, aliphatic, -onium, and heterocyclic compounds in which there was no nuclear iodine.

Archer and Suter (1952) have investigated a number of 1-alkyl- and 1-dialkylaminoalkylamino-thioxanthones (X) (cf. Miracil D) for testing as schistosomicides and found that chemical constitution also influenced the potency, e.g. it was discovered that the most effective derivatives, where $R_1 = R_2 = \underline{n}\text{-}C_4H_9$, were those with a methyl group in the 4-position and hydrogen, methyl, methoxy, or chlorine in the 7-position. Furthermore, it was found that, if the number of carbon atoms between the nitrogen atom in the side chain exceeded two, there was a very sudden fall in activity (cf. Mauss, 1948).



(X)

It/

It would appear, therefore, that there is some justification for believing that chemical constitution is a definite factor governing anthelmintic or larvicidal effect.

Methods of Testing. - The ideal method of estimating the value of possible anthelmintic substances would be to investigate the effect of these on the parasites in the host itself, a mode of procedure which is of very limited application for economic reasons. Even the use of laboratory animals would involve a great deal of expenditure of money and time, at any rate in the preliminary stages of the research. Consequently the development of suitable in vitro tests in the initial stages of anthelmintic investigation would be justifiable, whereby a large number and variety of chemical compounds could be 'screened' quickly and with the minimum of material against a wide range of helminths. Testing chemicals in vitro has the advantage that any paralysant or lethal effect can be compared without complications arising from the effect of chemicals on the host. Especially is this suitable when the anthelmintic effects of members of a homologous series are being compared, although in practice it is, of course, essential that anthelmintics be reasonably non-toxic to the host and not so nauseating that ruminants stop chewing the cud. It/

It is appreciated, however, that *in vitro* tests may be unable to detect a substance with anthelmintic properties and it may happen, on the other hand, that a compound which appears to have an anthelmintic effect in *in vitro* experiments might have no value whatsoever as an anthelmintic *in vivo* (cf. Erhardt, 1948), especially if it is rapidly changed in the host.

It is also recognised that anthelmintics tend generally to be selective in their action, e.g. some are only effective against nematodes, whilst others only attack cestodes (tapeworms). Moreover known anthelmintics produce very different results on related worms, even in the host, e.g. phenothiazine is very effective against *Haemonchus contortus* in the abamasum, but larger doses are required to remove *Ostertagia* spp. and *Trichostrongylus axei* from the abomasum, and other bursate nematodes from the small and large intestines. An extreme example is *Nematodirus* spp. on which phenothiazine seems to have no effect. In fact, there is evidence that after its use the number of *Nematodirus* spp. increases presumably because competition or some host resistance has been removed.

As indicated above, Baldwin (1948) attributed the conflicting results obtained with *in vitro* testing to unsatisfactory methods. Annelid worms, particularly the/

the earthworm and the leech, were frequently used in the earlier investigations (cf. Trendelenburg, 1915), but this was adversely criticised by Rebello, da Costa, and Rico (1928), Lamson and Ward (1936), Baldwin (1943), Baldwin and Moyle (1947, 1949), Duguid and Heathcote (1950a). Lamson and Ward, for example, examined the effect of a wide variety of compounds on earthworms and A. lumbricoides, but reported that no correlation of activity could be found. Duguid and Heathcote compared the effects of compounds on earthworms with those on tapeworms in vitro and found distinct differences. It is, therefore, emphasised that, if results are to be of any value, in vitro experiments must be carried out with several different genera or families of helminths.

von Schroeder (1885) experimented with entire helminths and tested them against various compounds. Rebello and Rico (1926) and Rico (1926) were able to obtain kymographic tracings with tied-off fragments of A. lumbricoides and da Costa (1931) used such preparations in studying the anthelmintic potencies of a number of organic compounds of arsenic. Lamson and Brown (1936), used whole ascarids when testing the anthelmintic properties of possible ascaricides. They exposed the worms to the action of the chemical at a certain concentration/

concentration and the time required to cause a lethal effect was taken as an index of activity. The temperature was maintained at 37.5°. The helminth was considered to be dead when no movement was observed when dropped into water at 60°.

Baldwin (1943) evolved a kymographic technique whereby chemicals in small quantities could be quickly tested against tied-off neuro-muscular preparations of A. lumbricoides. Two ligatured preparations could be made, viz., (1) an intermediate, obtained near the genital pore of the female and (2) an anterior, which contains the so-called 'nerve ring'. The preparation was placed in a vessel maintained at 38-39°; one end of the preparation was attached to a glass hook at the bottom of the vessel and the other to a lever with a writing point, the movements of which were recorded on a slowly revolving smoked drum. An upward movement of the writing point indicated contraction, whilst relaxation was shown when the point fell. If the point recorded no movement, the preparation was considered to be either paralysed or perhaps killed. A timing device was also incorporated and indicated the time in minutes. The movements of the preparation were recorded for at least 5 minutes, after it had been placed in Ringer's solution, and then the medium was rapidly/

rapidly replaced by either a solution, or emulsion using sodium glycocholate as the emulsifying agent, or a suspension of the compound under test at a definite concentration in Ringer's solution. Sodium glycocholate had no effect on the preparations. When a chemical caused cessation of movement of the preparation, it was difficult to say whether the chemical had a paralysant or lethal effect, because there is no known means of stimulating a merely paralysed ascarid preparation. Baldwin (1948) placed such a preparation, after treatment with the compound for 30 minutes, in fresh Ringer's solution, and observed whether there was any restoration of movement. However, if no movement was observed, one cannot be certain that there had been any lethal action. Baldwin decided to describe such effects as paralysant.

The method has its limitations and, as Baldwin pointed out, it is only suitable for compounds which act on the neuro-muscular mechanism of the nematode. Moreover, only a portion of the worm is used, which may seem a serious objection. On the other hand, it was found that these tied-off preparations could be kept alive for some considerable time. It was observed, for example, that several of these preparations could be kept for over 12 hours and appear to be quite active after that time.

To/

To have any effect on the preparation the chemical must penetrate the cuticle. Ability to penetrate the cuticle is a very important property since the entire A. lumbricoides, even with mouth closed, might be affected by the chemical. Baldwin (1948) criticised the use of entire A. lumbricoides as "not a reliable test object", since it had been observed by some workers that the anthelmintic santonin, which Baldwin (1943) found to be active against the anterior preparation of A. lumbricoides, was inactive towards the entire helminth. Baldwin found that, in general, substances known to possess anthelmintic properties gave positive results in his in vitro tests and no action was observed with those compounds which were inactive in in vivo experiments. There were, however, two apparent exceptions, viz., gentian violet, a successful anthelmintic for threadworm in man, and phenothiazine, an anthelmintic which is used extensively in the veterinary field against some bursate nematodes. However, the mode of action of gentian violet is unknown and that of phenothiazine still remains obscure although it, or one of its derivatives, is believed to possess a specific action on the reproductive organs. It has been found that the particle size of phenothiazine is an important factor.

This/

This technique had been extended to ascertain the action of chemicals on liver fluke (Fasciola hepatica) by Chance and Mansour (1949) and on the tapeworms, Moniezia expansa and Taenia saginata by Duguid and Heathcote (1950a, b.). Chance and Mansour used the entire liver fluke and, as the helminth was stimulated by amphetamine sulphate and strychnine hydrochloride, they were able to determine whether a compound was paralysant or lethal, on replacing the chemical under test after 45 minutes with solutions of either of these stimulants and observing whether the movement of the preparation was restored or not. If there had been cessation of movement due to the action of the compound under examination, and the movement of the worm restored on addition of amphetamine sulphate, the compound was assumed to have a paralysant effect on the preparation. No restoration of movement indicated a lethal effect. It was found that liver fluke was not only sensitive to all the compounds which affected A. lumbricoides, but also to a wider range of anthelmintics.

Duguid and Heathcote used pieces of Moniezia expansa and single segments of Taenia saginata. Strychnine hydrochloride, which had a stimulant effect, could be used to ascertain whether any cessation of movement,/

movement, caused by the chemical under investigation, was due to paralysis or death of the musculature. Marked differences were observed when the results were compared with those obtained with the same chemicals on A. lumbricoides by Baldwin (1943), possibly due to the helminths belonging to different phyla.

Seelkopf and Auterhoff (1950) tested chemicals against whole ascarids at 37°. If no movement of the parasite was observed on repeated shaking of the vessel and irradiation with a 40W. lamp for at least one hour, the chemical was considered to have a paralysant effect. No movement of the helminth after being placed in a pure physiological solution for about 4 hours indicated a lethal effect.

Wenzel and Gibson (1951) used the entire A. lumbricoides, which was secured at the posterior end to the bottom of the vessel, the temperature being maintained at 38 - 39°. The anterior end was attached to a lever which recorded the activity of the worm on a smoked kymograph drum. Only three chemicals were tested at a concentration of 1:1,000.

Kerr and Cavett (1952) have evolved a technique also using the entire helminth. The method is similar to Baldwin's, but Ascaridia galli, a nematode of maximum length 4 inches, was employed. They/

They maintained that the test appeared to be simpler than Baldwin's method. Examination of the tracings given in the paper, however, show the normal movements of the parasite to be irregular compared with the rhythmic movements obtained by Baldwin's technique.

Ueda et al. (1953) tested hydroxytetrahydronaphthalenes for anthelmintic activity by observing the time taken to produce a curling motion in A. lumbricoides. The percentage kills were calculated after 30 minutes exposure to a 1:1,000 solution of the compound in Ringer-Dale modified solution.

Leiper (1952) developed a method using the vinegar eelworm, Turbatrix aceti. Although this free-living nematode is not a parasite, it is closely related to the parasitic eelworms, e.g., the plant eelworms. This technique, which has not yet been published in detail, uses 50% vinegar as the medium. This is natural one for the nematode and serial dilutions of the compounds can be investigated. Leiper considers that this test has much to commend it and points out that in all probability it would be reliable for the testing of gastro-intestinal anthelmintics. Phenothiazine, as well as other anthelmintics, show up favourably with this technique. By its means a much/

much larger number of compounds can be screened in a given time than is possible with the other in vitro methods described above and very small quantities of the compounds are required.

Peters (1952) carried out a series of experiments on the culturing of vinegar eelworm in the laboratory and found that highly satisfactory cultures could be obtained using the media based on vinegar, sugar (4%), and ethanol (4%) at 24°.

The tests so far described are concerned with the adult helminth but, if means could be found to destroy the parasite in the early stages of its life history, the incidence of helminthiasis might be considerably reduced. Parnell (1936, 1938) devised a technique whereby chemicals were tested on the free-living stages of Sclerostomes (nematodes) from horse faeces with the view to evaluating the effect of various chemicals on them. In this method the number of larvae in a single test may amount to several thousand, so that in this respect it is better than those methods where comparatively few or only a single helminth is used. The technique, however may prove rather lengthy and require a comparatively large amount of chemical. The method is described in Paper No. 4 (p. 510).

Such/

Such are the better known *in vitro* methods of testing helminths, and compounds, found promising from these tests, could be tried out on infected laboratory animals before experimentation with domestic animals.

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PART II.

The research, which is the subject of this thesis, may be divided as follows:-

(1) The effect of substituent groups on anthelmintic potency.

(2) Studies of the anthelmintic properties of phenothiazine derivatives.

(3) Examination of miscellaneous chemical compounds for anthelmintic potency.

(4) The effects of various substances on the free-living stages of Sclerostomes (Nematoda).

The compounds used in (1), (2) and (3) were tested *in vitro* against *Ascaris lumbricoides* and liver fluke (*Fasciola hepatica*) belonging to the two phyla of helminths, viz., roundworms (nemathelminthes) and flatworms (platyhelminthes) respectively. These two species are large and easily obtainable. In addition they are suitable objects for *in vitro* testing and very common parasites.

Baldwin's kymographic technique (1943) was employed in (1), (2) and (3). All the other methods of/

of *in vitro* testing with *A. lumbricoides* were carefully considered and it was decided that the method chosen was the most suitable for the purpose and also the modification of the technique by Chance and Mansour (1949) for liver fluke was most convenient. This technique is described in detail in Part I of this thesis.

Considering that living material was used, the method gave remarkably consistent results when tests were repeated, and experiments could be carried out expeditiously.

Leiper's vinegar eelworm technique was also studied, and since it appeared to be very promising as a rapid screening test, further details were required, which Mr. J. W. G. Leiper supplied. The ease with which the eelworm could be cultured in the laboratory was an additional advantage (cf. Leiper, 1952; Peters, 1952). The technique was improved upon and subsequently used in (1), (2) and (3) (see Paper No. 15).

Cultures of *Rhabditis pellio* were made by methods similar to those of Johnson (1913), and Dougherty and Calhoun (1948), but attempts to test the cultures against chemicals with a view to ascertaining their *in vitro* anthelmintic properties were unsuccessful.

Observations/

Observations on the effect of various substances on the free-living stages of Sclerostomes were considered important, since these are pre-adult forms and they can be cultured easily in the laboratory. Tests were carried out using Parnell's technique, described in Paper No. 4, (p. 510).

The Effect of Substituent Groups on Anthelmintic Potency. - As indicated in Papers Nos. 1, 3, and 5, 2:3-dihydro-3-ketobenzo-1:4-thiazine and derivatives might possibly show anthelmintic properties as revealed by in vitro testing on account of certain structural similarities to substances with known anthelmintic properties.

The preparation of these compounds is described in Papers Nos. 1, 5, and 8. Derivatives with substituents in the 6-position were the most readily obtainable and served the purpose for varying the substituents in the molecule, whilst the ring containing the keto-group remained unaltered. This allowed a study to be made of the effects of certain atoms or groups on the anthelmintic potency of the unsubstituted compound and made possible the arrangement of the substituents in order of potency.

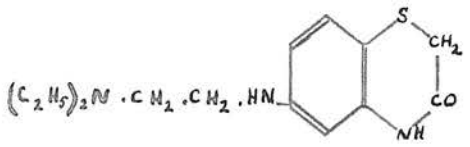
After several attempts the 6-bromo-derivative was obtained analytically pure by the method described in/

in Paper No. 8. There is no apparent reason why a pure sample of this compound should not have been obtained either by Sandmeyer reaction or by decomposition of the diazonium perbromide, since the other halogen derivatives were comparatively easily obtained by the usual methods. It may be that the 6-position is not particularly reactive, as the cyanogen group could not be introduced by Sandmeyer reaction, the 6-iodo-derivative would not form a Grignard reagent, and an attempt to carry out a Skraup synthesis from the amino-derivative proved fruitless. The exceptionally long time required for complete acetylation of the amino-group (16 hrs.) is noteworthy. The imino-group in the second ring is not attacked.

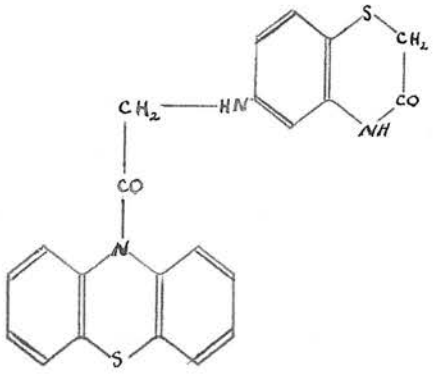
Attempts to condense the 6-chloro-derivative with 2-diethylaminoethylamine and the 6-amino-derivative with 10-chloroacetylphenothiazine, and to prepare the 6:7-dichloro-derivative were unsuccessful (Paper No. 8).

It was considered that the introduction of the diethylaminoethylamino-group into the 2:3-dihydro-3-ketobenzo-1:4-thiazine molecule might give a compound/

compound (XI) with anthelmintic properties, since Mauss and collaborators (1946, 1948) found schistosomicidal activity with certain xanthone and thioxanthone derivatives containing a diethylamino-ethylamino-group (this thesis, p.8).



(XI)



(XII)

Ekstrand (1949) obtained 10-acylamino-derivatives of phenothiazine by reaction of halogeno-acyl halides with phenothiazine dissolved in benzene and heating the resulting 10-halogeno-acylphenothiazines with amines in benzene solution in a sealed tube to 70°. A compound with phenothiazine and 2:3-dihydro-3-ketobenzo-1:4-thiazine residues such as (XII), might be/

be expected to show some anthelmintic activity.

Attempts to prepare 6:7-dichloro-2:3-dihydro-3-ketobenzo-1:4-thiazine were made in order to compare its in vitro anthelmintic properties with those of the 6-chloro-derivative. Treatment of the dihydroxy derivative with phosphorus pentachloride or thionyl chloride gave a deep violet coloured compound.

Paper No. 10 describes the preparation of rhodanine derivatives, with probable anthelmintic properties. Incidentally the investigation afforded some interesting structural problems. Some compounds containing both rhodanine and phenothiazine residues were prepared.

The methods for the preparation of thiazoles and benzothiazoles as possible anthelmintics are given in Papers Nos. 11 and 12. Most of the derivatives in Paper No. 11 contain phenothiazine residues. A method of obtaining 6-substituted-2-mercaptobenzothiazoles in good yield, and a mechanism for their formation are indicated in Paper No. 12.

Paper No. 3 describes the action of 2:3-dihydro-3-ketobenzo-1:4-thiazine and derivatives against liver fluke. The following were tested against liver/

liver fluke and also the anterior preparations of A. lumbricoides:- azo-dyestuffs derived from 6-amino-2:3-dihydro-3-ketobenzo-1:4-thiazine (Paper No. 5); derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, and of rhodanine (Paper No. 14); benzothiazoles (Paper No. 16). The effect of these various compounds on vinegar eelworms is discussed in Paper No. 15.

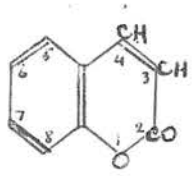
The investigation of the anthelmintic properties of derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, of rhodanine, and of benzothiazole, have shown that certain substituents do affect the anthelmintic activity of a compound, as indicated by in vitro tests, and that such substituents can be arranged in order of potency.

Studies of the Anthelmintic Properties of Phenothiazine Derivatives. - It was considered that this might be a fruitful line of investigation since phenothiazine is employed to a considerable extent as an anthelmintic in veterinary practice, although certain disadvantages attend its use. Paper No. 7 describes a simple method for the detection of phenothiazine, and its application in a case of phenothiazine poisoning. An important review on the anthelmintic properties of phenothiazine has been published recently by Harwood (1953).

As a number of oxidation products have been found in the host dosed with phenothiazine, e.g. phenothiazone, thionol, or their leuco-compounds, and phenothiazine sulphoxide, these three compounds were prepared and tested against liver fluke and A. lumbricoides in vitro as described in Papers Nos. 2 and 6 respectively. The three compounds have definite anthelmintic properties as far as can be demonstrated by in vitro experiments. Phenothiazone is outstanding, not only because of its lethal effect on liver fluke, but also its paralysant or perhaps lethal effect on A. lumbricoides. Liver fluke was stained by a 1:1,000 suspension of phenothiazone, but only the vitelline glands, and the muscle around the oral and ventral suckers. No reproductive organs were stained, even after 1 hour.

Phenothiazone is much more potent than thionol and the only apparent constitutional difference is the presence of the hydroxyl-group in position 7 in the latter compound. In this connection it is interesting/

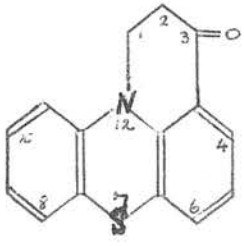
interesting to note that Baldwin (1943, 1948) found that coumarine (XIII) was active against the anterior preparation of A. lumbricoides, whilst umbelliferone (7-hydroxycoumarine) was inactive.



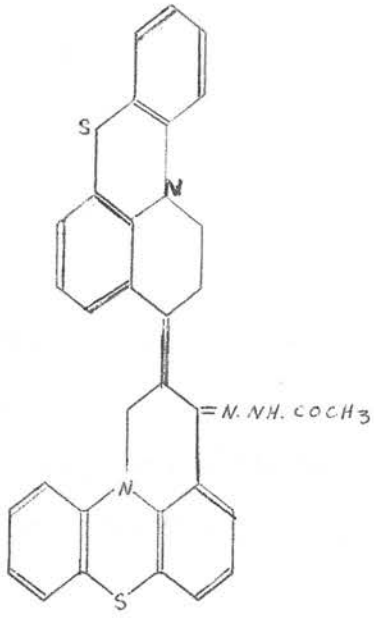
(XIII)

Other phenothiazine derivatives were prepared (Papers Nos. 9 and 13), with the view to obtaining improved anthelmintics of this type, and tested (Papers Nos. 14 and 15). Most of the compounds were substituted in the 10-position, since such derivatives are of increasing chemotherapeutic importance (cf. Massie, 1954). Some of the reactions described in Paper No. 13 gave unexpected products, especially in the reaction of Girard reagent P with 2:3-dihydro-3-oxo-1H-pyrid [3,2,1-kl]-phenothiazine (XIV)/

(XIII), in which two compounds were isolated, to one of which no definite structure could be assigned. Further investigation of the compound, however, showed it to have the structure (XV) (Paper No. 17). Another interesting reaction with 10-chloroacetylphenothiazine and thiourea is cited in Paper No. 12.



(XIV)



(XV)

Examination of Miscellaneous Chemical Compounds for Anthelmintic Potency. - About 80 miscellaneous compounds which had shown promise as Sclerostome larvicides, or probably might have proved interesting, were/

were tested *in vitro* (Papers Nos. 14 and 15). It was found that 80% of the compounds which affected *A. lumbricoides* were active against liver fluke, and the latter was the more sensitive helminth. In addition the liver fluke was affected by compounds which had no action against *A. lumbricoides*. Chance and Mansour (1949) record similar observations.

The Effects of Various Substances on the free-living Stages of Sclerostomes (Nematoda). - The results of this investigation are given and discussed in Paper No. 4, a joint publication with Dr. Parnell, who was entirely responsible for the helminthological work. The present author was responsible for the choice and preparation of many of the chemicals, and contributed the discussion on the chemical significance of the results.

For a comparison of the results of the four *in vitro* anthelmintic techniques used in the investigations described in this thesis, see Paper No. 18.

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The author is indebted to Dr. I. W. Parnell, late of Division of Helminthology, Department of Zoology, University of Edinburgh, for his collaboration in the research embodied in Papers Nos. 4 and 18; also to Mrs. G. M. Stewart, B.Sc., Drs. J. Raeburn, A. A. Cutler, and A. L. Misra, who have carried out much of the experimental work under the author's direction. All the preliminary experimentation, checking of results, and most of the work on phenothiazine derivatives were carried out by the author.

PUBLICATIONS

(1) Preparation of 2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives as Possible Anthelmintics.
J. chem. Soc., 1952, 787.

(2) The Effect of Some Oxidation Products of Phenothiazine on Liver Fluke (Fasciola hepatica) in vitro.
Brit. J. Pharmacol., 1952, 7, 215.

(3) The Influence of Groups in the Molecule of 2:3-Dihydro-3-ketobenzo-1:4-thiazine on its Effect on Liver Fluke (Fasciola hepatica) in vitro.
Brit. J. Pharmacol., 1952, 7, 219.

(4) Some Observations on the Lethal Effects of Various Chemicals against the Free-living Stages of Sclerostomes (Nematoda).
Brit. J. Pharmacol., 1952, 7, 509.

(5) Studies in Azo-Dyestuffs from 6-Amino-2:3-dihydro-3-ketobenzo-1:4-thiazine, with Special Reference to their Possible Anthelmintic Effects and Dyeing Properties.
Rec. Trav. chim. Pays-Bas, 1952, 71, 1198.

(6) The Effect of Some Oxidation Products of Phenothiazine on Ascaris lumbricoides in vitro.
Arch. int. Pharmacodyn., 1953, 92, 301.

(7) /

(7) A Simple Method for the Detection of Phenothiazine. Canad. J. comp. Med., 1953, 17, 377.

(8) Preparation of 2:3-Dihydro-3-oxobenzo-1:4-thiazine Derivatives as Possible Anthelmintics. Part II.
J. chem. Soc., 1953, 3716.

(9) Preparation of Phenothiazine Derivatives as Possible Anthelmintics.
J. chem. Soc., 1954, 2577.

(10) Preparation of Rhodanine Derivatives as Possible Anthelmintics.
J. chem. Soc., 1954, 3919.

(11) Preparation of Thiazoles and Benzothiazoles as Possible Anthelmintics.
J. chem. Soc., 1954, 4430.

Reprints of these publications (Nos. 1 - 11) are attached to the thesis.

The following papers have been accepted (Nos. 12 - 16, and 18), or submitted (No. 17) for publication as indicated (manuscripts attached):-

(12) The Preparation of Some Heterocyclic Sulphur Compounds as Possible Anthelmintics.
J. chem. Soc. (Reprint order No. 5709). Proof received.

(13)/

(13) Preparation of Phenothiazine Derivatives as Possible Anthelmintics. Part II.
J. chem. Soc. (Reprint order No. 5845).
Proof received.

(14) In vitro Tests of Chemical Compounds on Ascaris lumbricoides and Fasciola hepatica.
Brit. J. Pharmacol., 1955, 10, 222.
Proof received.

(15) In vitro Testing of Chemical Compounds against Vinegar Eelworm (Turbatrix aceti).
Attempted correlation of anthelmintic effect and chemical constitution.
Arch. int. Pharmacodyn. (Accepted for publication).

(16) In vitro Testing of Benzothiazoles and some Phenothiazine Derivatives against Ascaris lumbricoides and Liver Fluke (Fasciola hepatica).
Arch. int. Pharmacodyn. (Accepted for publication).

(17) An Abnormal Reaction with Girard Reagent P.
Chem. & Ind. (Submitted for publication).

(18) A Comparison of the Results of Four in vitro Anthelmintic Testing Techniques.
J. Pharm. Pharmacol. (Accepted for publication).

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PREPARATION OF 2:3-DIHYDRO-3-KETOBENZO-1:4-THIAZINE DERIVATIVES AS POSSIBLE ANTHELMINTICS

By

ALEXANDER MACKIE

AND

JOHN RAEBURN

Preprinted from the JOURNAL OF THE CHEMICAL SOCIETY
MARCH 1952, (142), *pages* 787—790

PAPER No. 1

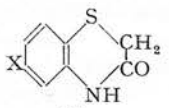
Preprinted from the Journal of the Chemical Society,
March 1952, (142), pages 787—790.

142. *Preparation of 2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine Derivatives as Possible Anthelmintics.*

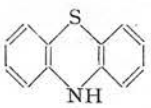
By Alexander Mackie and John Raeburn.

Derivatives of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine have been prepared, mainly from the diazonium compound from the 6-amino-derivative, for test against parasitic worms *in vitro*. Nearly all the derivatives produced a paralysant effect on liver fluke (*Fasciola hepatica*).

2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine (I; X = H) and some derivatives thereof have been prepared, as it was considered that they might show some anthelmintic properties : these compounds have some features of phenothiazine (II), extensively used in veterinary practice, and of filicic acid (III), an important constituent of *Filix mas*.



(I)



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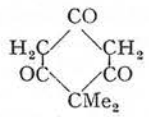

(III)

Few derivatives of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine have been synthesised. Friedlaender and Chwala (*Monatsh.*, 1907, **28**, 252) showed that when (*o*-nitroarylthio)-acetic acids were reduced, the resulting aminoarylthio-acids split off a molecule of water with extraordinary ease, with ring closure to benzo-1 : 4-thiazine derivatives.

2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and its 6 : 7-dimethoxy-derivative were obtained by reduction of the appropriate (nitrophenylthio)acetic acids (Claasz, *Ber.*, 1912, **45**, 751; Baldick and Lions, *J. Roy. Soc. N.S.W.*, 1937—38, **71**, 113).

Most of the derivatives described in this investigation were prepared by way of the diazonium compound from 6-amino-2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine (I; X = NH_2), obtained by reduction of (2 : 4-dinitrophenylthio)acetic acid (Friedlaender and Chwala, *loc. cit.*, p. 276). Many of the yields were small, principally owing to the difficulty in obtaining the compounds analytically pure. The amino-group was replaced successfully by F, Cl, I, CNS, N_3, NO_2, NO, SH, H_2AsO_3, H_2SbO_3, and HgCl, and decomposition of the diazonium compound by copper bronze afforded the bis-derivative. The 6-bromo-compound was not obtained by Sandmeyer reaction, but by decomposition of the perbromide. The 6-hydroxy-derivative could not be prepared from the diazonium compound. A deep red colour was obtained, which indicated self-coupling. The 6 : 7-dihydroxy-compound was, however, easily prepared from the 6 : 7-dimethoxy-derivative. The amino-group could not be replaced by the cyanogen group by Sandmeyer reaction, and attempts to produce a Grignard reagent from the iodo-derivative were fruitless.

The derivatives described herein have been tested against and had practically no effect on the round worm, *Ascaris lumbricoides*, but nearly all showed a paralysant effect on liver fluke (*Fasciola hepatica*) *in vitro*. Full details will be published elsewhere. The 6-chloro-derivative was the most effective.

Experimental

M. p.s are uncorrected.

6-*Amino*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—Reduction of (2 : 4-dinitrophenylthio)-acetic acid, m. p. 172° (Friedlaender and Chwala, *loc. cit.*, record m. p. 167—168°), with tin and hydrochloric acid afforded the 6-amino-compound. Contrary to the findings of Friedlaender and Chwala (*loc. cit.*) who preferred using iron and acetic acid, the former reagents proved superior. Reduction in alcohol with iron and hydrochloric acid (West's method, *J.*, 1925, **127**, 494) was unsatisfactory.

The hydrochloride decomposed at 272—274°, and the acetyl derivative had m. p. 257°. Friedlaender and Chwala (*loc. cit.*) give m. p. 257° for the latter compound, but record few experimental details. Satisfactory yields were only obtained after 16 hours' refluxing with acetic anhydride.

The amino-compound was easily diazotised below 10°, and for each of the derivatives prepared by way of the diazo-reaction, 4·5 g. of amino-derivative were used.

6-*Fluoro*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—Fluoroboric acid (40% solution; 20 c.c.) was added to the cold diazonium solution. The mixture was stirred for 2 hours, a pale yellow precipitate of the diazonium fluoroborate separating, which was filtered off, washed with water, ethanol, and ether, and finally dried (6 g.). The diazonium fluoroborate was decomposed at 135° (vapour from boiling xylene) and the 6-*fluoro*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine* in the residue was extracted with boiling absolute ethanol. An orange-brown impurity, which separated when the alcoholic extract cooled, was removed, and the fluoro-compound in the filtrate was isolated and recrystallised from aqueous ethanol in pale yellow platelets (1 g.), m. p. 184° (Found : C, 52·4; H, 3·2; N, 7·7. C_8H_6ONFS requires C, 52·5; H, 3·3; N, 7·7%).

6-*Chloro*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—The amino-group was replaced by chlorine in a Sandmeyer reaction (Friedlaender and Chwala, *loc. cit.*).

6-*Bromo*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—Decomposition of the perbromide at 155° afforded a compound, m. p. 220° (Found : N, 5·0. Calc. for C_8H_6ONBrS : N, 5·7%). A purer product could not be obtained.

6-*Iodo*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—A saturated aqueous solution of potassium iodide (8 g.) was added to the cold diazonium solution. The reaction mixture was stirred during 5 hours, then refluxed for 1 hour with ethanol (200 c.c.), and the solution filtered hot. The crystals obtained on cooling of the filtrate were recrystallised from aqueous ethanol, giving the 6-*iodo*-compound as feathery orange needles (3·5 g.), m. p. 208—210° (Found : I, 43·5; N, 5·1. C_8H_6ONIS requires I, 43·6; N, 4·8%).

2 : 3-*Dihydro*-3-*keto*-6-*thiocyanatobenzo*-1 : 4-*thiazine*.—Potassium thiocyanate (4 g.) in aqueous solution, followed by a paste containing cuprous thiocyanate, was added to the cold diazonium solution. The paste was prepared by adding potassium thiocyanate (4 g.) in aqueous solution to a solution containing copper sulphate (8 g.) and ferrous sulphate (16 g.), filtering, and making the residue into a paste with water. The mixture was vigorously agitated for 9 hours, then the reaction product was refluxed with ethanol (200 c.c.) for 1·5 hours. After filtration, the filtrate deposited orange crystals which were recrystallised from aqueous ethanol. 2 : 3-*Dihydro*-3-*keto*-6-*thiocyanatobenzo*-1 : 4-*thiazine* was obtained as pale yellow needles (3 g.), m. p. 180° (Found : C, 48·4; H, 3·1; N, 12·3. $C_9H_6ON_2S_2$ requires C, 48·6; H, 2·7; N, 12·6%).

6-*Azido*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—Sodium azide (2 g.) in aqueous solution was slowly added to the cold diazonium solution. Nitrogen was evolved and a white precipitate formed. The mixture was stirred below 10° for 5 hours, and then allowed to attain room temperature. The precipitate was filtered off, washed, and recrystallised from aqueous ethanol. 6-*Azido*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine* was obtained as pale yellow feathery needles (5 g.), becoming yellowish-brown with slight decomposition on exposure to light, m. p. 176° (decomp.) (Found : N, 27·0. $C_8H_6ON_4S$ requires N, 27·2%).

2 : 3-*Dihydro*-3-*keto*-6-*nitrobenzo*-1 : 4-*thiazine* (cf. Hodgson and Marsden, *J.*, 1944, 22).—The diazonium solution was neutralised with calcium carbonate and filtered. Finely powdered sodium cobaltinitrite (11 g.) was then added to the filtrate and the diazonium cobaltinitrite which separated was filtered off and dried. The diazonium cobaltinitrite (10 g.) was added in portions at room temperature to an aqueous solution containing sodium nitrite (10 g.) and copper sulphate (10 g.), in which cuprous oxide (4 g.) was suspended. After evolution of nitrogen was complete, the product was filtered off. The greenish residue was recrystallised from ethanol, giving the 6-*nitro-thiazine* as golden-brown feathery needles (1 g.), m. p. 243—244° (Found : N, 13·6. $C_8H_6O_3N_2S$ requires N, 13·3%).

2 : 3-*Dihydro*-3-*keto*-6-*nitrosobenzo*-1 : 4-*thiazine*.—The diazonium solution was run into a saturated solution of potassium permanganate (70 c.c.) below 10° and the mixture rendered alkaline with aqueous potassium hydroxide. After 3 hours' stirring, the mixture was extracted with ether, and on removal of the ether the residue was recrystallised from aqueous ethanol, giving 2 : 3-*dihydro*-3-*keto*-6-*nitrosobenzo*-1 : 4-*thiazine* as pale yellow platelets (0·2 g.), m. p. 134° (Found : N, 14·7. $C_8H_6O_2N_2S$ requires N, 14·4%).

2 : 3-*Dihydro*-3-*keto*-6-*mercaptobenzo*-1 : 4-*thiazine*.—Aqueous potassium xanthate (4 g.) was added to the cold diazonium solution. After 2 hours, a bright yellow precipitate was obtained, the temperature of the reaction mixture was gradually raised, and decomposition com-

pleted on the water-bath (1·5 hours). The yellow precipitate was filtered off and refluxed with alcoholic potassium hydroxide until hydrolysis of the ethyl thiocarbonic acid derivative was complete (1 hour). After removal of the ethanol, the residue was dissolved in water, and the solution filtered and acidified with dilute sulphuric acid. The yellow precipitate was filtered off and recrystallised from aqueous ethanol; the *mercapto-thiazine* was obtained as pale yellow needles (3 g.), m. p. 174° (Found: C, 48·9; H, 3·2; N, 7·4. $C_8H_7ONS_2$ requires C, 48·7; H, 3·6; N, 7·1%).

2 : 3-*Dihydro*-3-*ketobenzo*-1 : 4-*thiazine*-6-*arsonic Acid*.—A solution of sodium arsenite (25 c.c.; 20%) was added to the cold diazonium solution, and the mixture rendered alkaline with aqueous sodium hydroxide. After being stirred (3—4 hours), the solution was filtered, the filtrate acidified and boiled with animal charcoal, and the purified filtrate made strongly acid. Brownish crystals were obtained on cooling and were recrystallised from hot water. The *arsonic acid* formed pale yellow plates (0·5 g.), decomp. >300° (Found: C, 33·3; H, 2·7; N, 5·0. $C_8H_8O_4NSAs$ requires C, 33·2; H, 2·8; N, 4·8%).

2 : 3-*Dihydro*-3-*ketobenzo*-1 : 4-*thiazine*-6-*stibonic Acid*.—A solution of antimony trioxide (8 g.) in concentrated hydrochloric acid, added to the ice-cold diazonium solution, gave the *stibonic acid*. This was purified by dissolution in aqueous sodium carbonate and reprecipitation with acid, and then formed a reddish-brown amorphous powder (1 g.), decomp. >270° (Found: C, 28·8; H, 2·5; N, 3·9. $C_8H_8O_4NSSb$ requires C, 28·6; H, 2·4; N, 4·2%).

6-*Chloromercuri*-2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazine*.—The diazonium solution was added to a solution of mercuric chloride (7 g.) in concentrated hydrochloric acid (7 c.c.) mixed with ice (7 g.). After 2 hours' stirring at 0°, the diazonium mercurichloride was filtered off, dried, and decomposed with copper bronze (6 g.) in presence of acetone (80 c.c.) at 0° (3 hours). The temperature was then raised slowly and decomposition completed on the water-bath (1 hour). On cooling, the product was filtered off and recrystallised from nitrobenzene. The *chloromercuri-thiazine* was obtained as pale yellow needles (1 g.), m. p. 263—264° (decomp.) (Found: C, 24·3; H, 1·2; N, 3·4. $C_8H_6ONClSHg$ requires C, 24·0; H, 1·5; N, 3·5%).

Bis-(2 : 3-*dihydro*-3-*ketobenzo*-1 : 4-*thiazin*-6-*yl*).—An aqueous alcoholic solution of the diazonium compound was decomposed with copper bronze (5 g.) introduced slowly, the temperature being kept below 30°. After 1 hour, the temperature was raised gradually to 75°, whereupon a vigorous reaction took place, and the resulting reddish-brown precipitate was filtered off. Excess copper was removed, and the residue was purified by refluxing it with absolute ethanol. The *dithiazinyl* was obtained as a brown amorphous powder (3·5 g.), m. p. >330° (Found: N, 8·3. $C_{16}H_{12}O_2N_2S_2$ requires N, 8·5%).

2 : 3-*Dihydro*-6 : 7-*dihydroxy*-3-*ketobenzo*-1 : 4-*thiazine*.—2 : 3-Dihydro-3-keto-6 : 7-dimethoxybenzo-1 : 4-thiazine (5 g.) (Baldick and Lions, *loc. cit.*) was refluxed with a large excess of constant-boiling hydriodic acid for 2 hours. On removal of methyl iodide and addition of a large volume of water, brown crystals separated, which were purified by recrystallisation from distilled water out of contact of air. The *dihydroxy-thiazine* crystallised as pale pink rectangular plates (1 g.), decomp. >240°, becoming deep pink on exposure to air. A purple colour, changing quickly to sky blue, developed with aqueous sodium hydroxide, but no colour with ferric chloride (Found: C, 48·8; H, 3·2; N, 7·0. $C_8H_7O_3NS$ requires C, 48·7; H, 3·6; N, 7·1%).

The authors' thanks are due to Principal Nisbet for his interest in this work and to Dr. J. W. Minnis and Mr. A. T. Macdonald for the microchemical analyses. Financial support from the Agricultural Research Council is gratefully acknowledged.

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THE EFFECT OF SOME OXIDATION PRODUCTS OF PHENOTHIAZINE ON LIVER FLUKE *(FASCIOLA HEPATICA) IN VITRO*

BY

ALEXANDER MACKIE and JOHN RAEBURN

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THE EFFECT OF SOME OXIDATION PRODUCTS OF PHENOTHIAZINE ON LIVER FLUKE (*FASCIOLA HEPATICA*) *IN VITRO*

BY

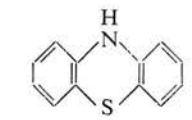
ALEXANDER MACKIE AND JOHN RAEBURN

From the Department of Chemistry, Heriot-Watt College, Edinburgh

(Received November 23, 1951)

Phenothiazine (I) is used extensively as an anthelmintic and has proved to be very efficient in the removal of some intestinal worms in sheep. A considerable amount of research has been carried out in order to ascertain the fate of phenothiazine when administered to ruminants (Collier, 1940 ; Swales and Collier, 1940 ; Lipson, 1940 ; Collier, Allen, and Swales, 1942 ; Collier, Allen, and Swales, 1943 ; Clare, 1947 ; Harpur, Swales, and Dunsteht, 1950).

The dose of phenothiazine required appeared to be unnecessarily large when its insolubility was considered, and Taylor and Sanderson (1940) studied the possibility of the formation in the host of another substance of greater anthelmintic potency than phenothiazine.



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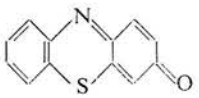

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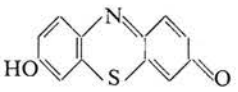

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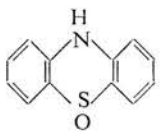

(IV)

A number of oxidation products have been found in the host dosed with phenothiazine, viz., phenothiazone (II), thionol (III), or their easily oxidizable leuco-compounds, phenothiazine sulphoxide (IV), and certain conjugates, e.g., leuco-phenothiazone conjugated with sulphuric acid.

Phenothiazone was administered to sheep by Collier, Allen, and Swales (1943), but found to have no effect on nematodes removable by phenothiazine, and both phenothiazone and thionol were tested by Taylor and Sanderson (1940) in goats heavily infected with parasitic worms, but no anthelmintic effect was observed.

As no mention was made of the liver fluke (*Fasciola hepatica*) in these experiments, the investigation described in the present paper was carried out to ascertain the action, if any, of phenothiazone, thionol, and phenothiazine sulphoxide on liver fluke *in vitro*.

METHODS

Preparative.—Phenothiazone, thionol, and phenothiazine sulphoxide were prepared by the methods of Olivier and Combé (1950), Houston, Kester, and DeEds (1949), and Barnett and Smiles (1909) respectively.

Biological testing.—The liver fluke was tested *in vitro* by the kymographic technique of Baldwin (1943) as modified by Chance and Mansour (1949). By frequent changes of the Ringer solution (1.5–2 hr.), it was possible to keep the liver fluke in a lively condition for 7 to 8 hr., after removal from the host. Aeration of the Ringer solution had no effect on prolonging the life of the parasite.

The tests were carried out at 37–38° C. with concentrations of 1 : 1,000 and less till the phenothiazine oxidation products ceased to have an effect on the helminth. As the compounds under test were very insoluble in water, emulsification by Baldwin's method was attempted. Phenothiazine sulphoxide formed only a suspension at the concentrations used.

RESULTS

It was found that phenothiazone had a lethal effect from a concentration of 1 : 1,000 to 1 : 8,000 and a paralysant effect from 1 : 8,000 to 1 : 16,000, whilst thionol and phenothiazine sulphoxide had no lethal action, and were paralysant at 1 : 1,000 and from 1 : 1,000 to 1 : 4,000 respectively.

Figs. 1–4 show the kymographic records of the three compounds at the minimum concentration required to produce a lethal or paralysant effect. The time is marked in minutes on the signal line, and the movement of the helminth in the Ringer solution is shown from the beginning of the experiment to the first long stroke, when the Ringer solution was replaced by the emulsion or suspension of the compound. At the second long stroke on the signal line, amphetamine sulphate (1 : 5,000) in Ringer's solution, as recommended by Chance and Mansour (1949), replaced the compound. Restoration of the movement of the parasite with amphetamine sulphate indicates that the compound had a paralysant effect, whilst no movement shows that the substance was lethal.

The results are summarized in Table I.

TABLE I

THE LOWEST CONCENTRATIONS AT WHICH OXIDATION PRODUCTS OF PHENOTHIAZINE EXERT A LETHAL OR PARALYSANT EFFECT ON THE LIVER FLUKE *in vitro*

Compound	Nature of preparation	Concentration	Effect
Phenothiazone	Emulsion	1 : 8,000	Lethal
	,,	1 : 16,000	Paralysant
Thionol	,,	1 : 1,000	,,
Phenothiazine sulphoxide	Suspension	1 : 4,000	,,

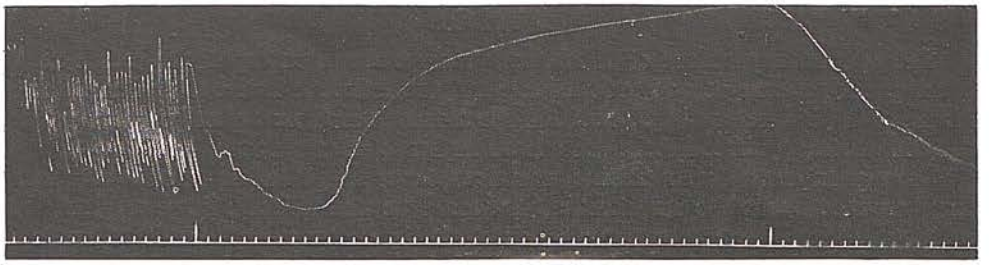

FIG. 1.—Lethal effect of phenothiazone (1 : 8,000). No response to amphetamine sulphate.

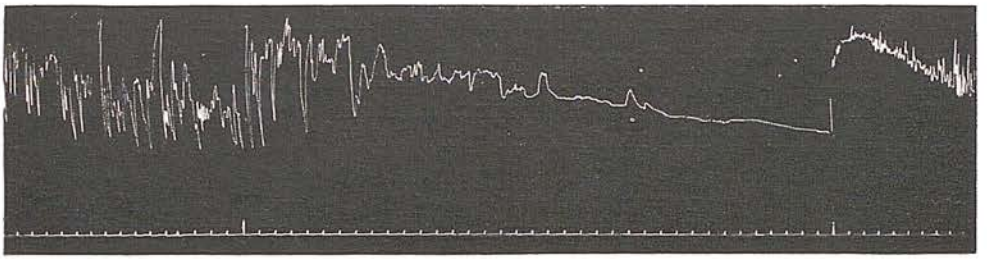

FIG. 2.—Paralysant effect of phenothiazone (1 : 16,000) after 25 min., followed by response to amphetamine sulphate.

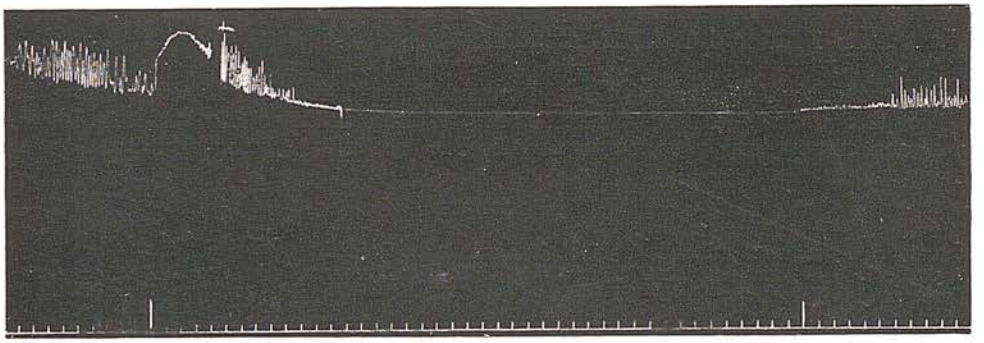

FIG. 3.—Paralysant effect of thionol (1 : 1,000), followed by response to amphetamine sulphate.

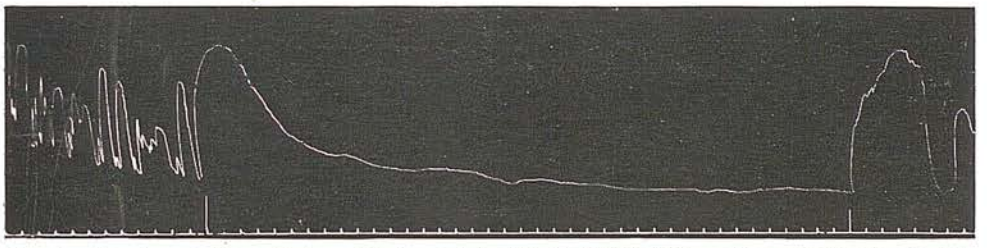

FIG. 4.—Paralysant effect of phenothiazine sulphoxide (1 : 4,000), followed by response to amphetamine sulphate.

Discussion

The outstanding feature of this investigation is the discovery of the lethal effect of the phenothiazone at 1 : 8,000 and its paralysant effect at 1 : 16,000. Chance and Mansour (1949) also found that certain substances, which were paralysants at lower concentrations, produced lethal effects at higher concentrations.

As a paralysant phenothiazone is sixteen times as potent as thionol and four times as effective as the sulphoxide. It should be pointed out, however, that the potency of phenothiazine sulphoxide is not strictly comparable with the other two compounds, since an effective concentration was only achieved by using a suspension.

It would appear, therefore, that the introduction of a hydroxyl group in position 7 of the phenothiazone molecule reduces its paralysant effect to one-sixteenth of its value and destroys its lethal effect.

It is significant that phenothiazine has no paralysant or lethal effect *in vitro* (Chance and Mansour, 1949), and therefore additional experiments *in vivo* with phenothiazine and its oxidation products would be desirable.

Summary

1. Phenothiazone, thionol, and phenothiazine sulphoxide, oxidation products of phenothiazine, have been prepared and tested at various concentrations against liver fluke (*Fasciola hepatica*) *in vitro*.

2. Phenothiazone had a lethal effect at a concentration of 1 : 8,000 and was paralysant at 1 : 16,000. Thionol and phenothiazine sulphoxide had only paralysant effects at 1 : 1,000 and 1 : 4,000 respectively.

We are indebted to the Agricultural Research Council for a grant which defrayed the cost of this investigation and to Mr. Soulsby, of the Edinburgh City Abattoir, for the collection of parasites. Our thanks are also due to Dr. Parnell for his interest in this investigation.

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THE INFLUENCE OF GROUPS IN THE MOLECULE OF 2 : 3-DIHYDRO-3-KETOBENZO-1 : 4-THIAZINE ON ITS EFFECT ON LIVER FLUKE *(FASCIOLA HEPATICA) IN VITRO*

BY

ALEXANDER MACKIE and JOHN RAEBURN

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THE INFLUENCE OF GROUPS IN THE MOLECULE OF 2 : 3-DIHYDRO-3-KETOBENZO-1 : 4-THIAZINE ON ITS EFFECT ON LIVER FLUKE (*FASCIOLA HEPATICA*) *IN VITRO*

BY

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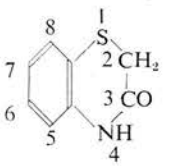
(Received January 17, 1952)

The parent compound chosen for the investigation described in this paper was 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine (I), as it contains in its molecule certain features present in substances which possess anthelmintic properties, e.g. phenothiazine (II). It also contains a $-CH_2CO-$ unit, which is a characteristic feature in filicic acid (III), the most important of the organic acids present in the anthelmintic *Filix mas*.



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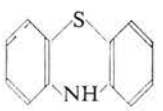

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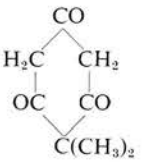


(III)

On account of these similarities, it was reasonable to assume that 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine might show some anthelmintic activity, and consequently this compound and a number of its derivatives with substituents in the 6- and 6 : 7-positions were tested against liver fluke (*Fasciola hepatica*) *in vitro*, so that the influence of the various groups on anthelmintic activity could be compared.

METHODS

Preparative.—2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and derivatives were prepared by methods described elsewhere (Mackie and Raeburn, 1952). Unfortunately it was not possible to prepare the 6-bromo-derivative analytically pure, so that a complete comparison of the influence of the four halogens could not be made.

Biological testing.—The method of *in vitro* testing adopted was Baldwin's kymographic technique (1943) as modified by Chance and Mansour (1949) for liver fluke.

The maximum concentration used was 1 : 1,000, either as solution, emulsion, or suspension, and the tests were carried out at lower concentrations till no effect on the helminth was observed. The minimum effective concentration was noted for each compound, so that it was possible to arrange most of the substituent groups in order according to their effect on potency. There were, however, a few compounds whose minimum effective concentration could not be determined owing to their insolubility, for even at 1 : 4,000 heavy suspensions were obtained.

At least four determinations were carried out on every compound at each concentration, unless the compound had no effect, when two tests were considered sufficient. Moreover, two or sometimes three determinations were carried out at the same concentration for a given compound with different batches of parasites. Except in a very few determinations, the kymographic records for a given compound at a particular concentration were remarkably similar in form.

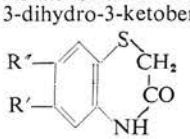
Results and Discussion

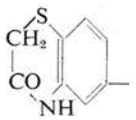
2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and its derivatives, which have been fully tested *in vitro*, have shown a paralysant effect on liver fluke. The most potent was the 6-chloro-derivative, which produced a paralysant effect at 1 : 8,000, the minimum effective concentration. At a concentration of 1 : 1,000 the 6-amino- and 6-mercapto-compounds had so powerful an effect that the addition of amphetamine produced only small irregular movements after 10 min. At 1 : 2,000, the 6-amino-derivative produced a paralysant effect for about 20 min., which was then followed by small convulsive movements.

The 6-arsonic and -stibonic acids, the 6-chloromercuri-derivative, and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) were very insoluble and the minimum

TABLE I

The minimum effective concentration is the lowest at which a paralysant effect was observed.
Derivatives of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine



Compound	R′	R″	Nature of preparation	Minimum effective concentration
2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine	H	H	Emulsion	1 : 2,000
6-Amino- ,, ,, ...	NH_2	H	,,	1 : 1,000
6-Amino- ,, ,, HCl	NH_2,HCl	H	Solution	1 : 3,000
6-Acetoamino- ,, ,, ...	$CH_3CO.NH$	H	Suspension	1 : 1,000
6-Fluoro- ,, ,, ...	F	H	,,	1 : 2,000
6-Chloro- ,, ,, ...	Cl	H	Emulsion	1 : 8,000
6-Iodo- ,, ,, ...	I	H	Suspension	1 : 5,000
6-Thiocyano- ,, ,, ...	CNS	H	,,	1 : 1,000
6-Triazo- ,, ,, ...	N_3	H	Emulsion	1 : 6,000
6-Nitro- ,, ,, ...	NO_2	H	,,	1 : 4,000
6-Nitroso- ,, ,, ...	NO	H	,,	1 : 6,000
6-Mercapto- ,, ,, ...	SH	H	,,	1 : 1,000
6-Arsonic Acid ,, ,, ...	H_2AsO_3	H	Suspension	Not obtained
6-Stibonic Acid ,, ,, ...	H_2SbO_3	H	,,	,, ,,
6-Chloromercuri- ,, ,, ...	HgCl	H	,,	,, ,,
6 : 7-Dimethoxy- ,, ,, ...	CH_3O	CH_3O	Emulsion	1 : 4,000
6 : 7-Dihydroxy- ,, ,, ...	OH	OH	,,	1 : 1,000
Bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl)		H	Suspension	Not obtained

effective concentration was not obtained. At a concentration of 1 : 4,000, the 6-chloromercuri-compound had a powerful depressant action but was not completely paralysant, the 6-arsonic acid showed a slight depressant effect, but the 6-stibonic acid and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) had no effect.

The results are summarized in Table I.

A typical kymographic record is shown in Fig. 1. This illustrates the effect of the compound on the helminth. The normal rhythmic movement of the worm in Ringer's solution at 37–38° C. is shown from the beginning of the experiment to the first long stroke on the signal line marked in minutes, when an emulsion of the compound under test replaced the original Ringer's solution. Amphetamine sulphate (1 : 5,000) in Ringer's solution, as employed by Chance and Mansour (1949), replaced the compound after approximately 45 to 50 min., as indicated by the second long stroke on the signal line. Restoration of the movement of the parasite on addition of amphetamine sulphate indicated that the compound was paralysant.

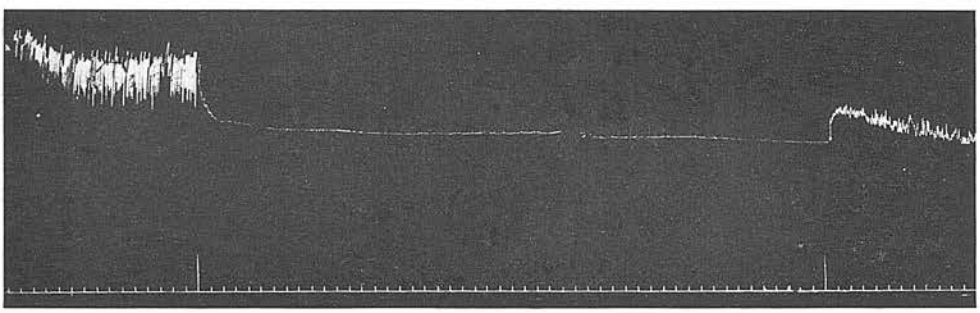


FIG. 1.—Paralysant effect of 6-chloro-2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine (1 : 8,000), followed by response to amphetamine sulphate (1 : 5,000).

In the following remarks it should be pointed out that comparisons between solutions, emulsions, and suspensions are not strictly accurate, but may be considered to be approximate (cf. Baldwin, 1943).

The introduction of the amino-group lowers the anthelmintic potency of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine by one half, but the amino-hydrochloride is three times more effective than the free base ; this difference may, however, be due to free acid in solution. Acetylation does not appreciably alter the potency of the base. This is perhaps surprising, as acetylation might be expected to effect some change.

With the exception of fluorine, which does not affect the activity of the unsubstituted compound, the introduction of halogen atoms (Cl or I) increases its potency. The chloro-derivative is four times as effective as the unsubstituted compound, the order of potency of the halogens being Cl>I>F.

The triazo- and nitroso-groups also have a considerable influence on anthelmintic activity. It is particularly interesting to note that the latter group is 1.5 times as effective as the nitro-group.

The thiocyano- and mercapto-groups both halve the activity of the unsubstituted compound.

The anthelmintic potency of the unsubstituted compound is increased twofold by the introduction of two methoxy-groups in the 6 : 7-positions. The corresponding dihydroxy-derivative, however, is only half as effective as the unsubstituted compound ; this was unexpected, as many examples are known where the introduction of a hydroxy-group increases the activity. It is possible that the vicinal position of the hydroxy-groups may be a controlling factor.

The very insoluble derivatives, the 6-arsonic and 6-stibonic acids, the 6-chloromercuri-compound, and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) might have had a paralysant effect at concentrations higher than 1 : 4,000 if they had been more soluble.

From the results obtained, it will be seen that the order of potency of the radicals is as follows : $Cl > N_3, NO > I > NO_2$, 6 : 7-dimethoxy $> NH_2, HCl >$ unsubstituted, $F > NH_2$, $CH_3.CO.NH$, CNS, SH, 6 : 7-dihydroxy.

The results of testing 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine and its derivatives suggest that the constitutional similarities of this compound to phenothiazine and to filicic acid, already noted, have some significance. Although Chance and Mansour (1949) found phenothiazine to be without effect on liver fluke *in vitro*, they did find that *Filix mas* was lethal at a concentration of 1 : 5,000.

Summary

1. 2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and derivatives with substituents in the 6- and 6 : 7-positions were tested against liver fluke (*Fasciola hepatica*) *in vitro* and the effects recorded kymographically.

2. All the compounds, which could be tested over a wide range of concentrations, showed paralysant effects.

3. Minimum effective concentrations (MEC) were estimated and it was possible to arrange the substituent groups in the following order of potency (MEC in parentheses) : $Cl(1 : 8,000) > N_3$, $NO(1 : 6,000) > I(1 : 5,000) > NO_2$, 6 : 7-dimethoxy (1 : 4,000)$> NH_2HCl(1 : 3,000)$>unsubstituted compound, $F(1 : 2,000) > NH_2$, $CH_3.CO.NH$, CNS, SH, 6 : 7-dihydroxy (1 : 1,000).

4. The 6-amino- and 6-mercapto-derivatives were paralysant at a concentration of 1 : 1,000, and severely reduced the subsequent response to amphetamine sulphate.

5. Owing to the insolubility of the 6-arsonic acid, 6-stibonic acid, 6-chloromercuri-derivatives and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl), no minimum effective concentration could be obtained, but a 1 : 4,000 suspension of the 6-chloromercuri-derivative produced almost complete paralysis ; the 6-arsonic acid was slightly depressant and the other two derivatives without effect at this concentration.

It is a pleasure to acknowledge our indebtedness to the Principal and Governors of the Heriot-Watt College for providing the laboratory facilities for this investigation and to the Agricultural Research Council for financial aid. Our thanks are also due to Professor Bell for his interest in this work, to Mr. A. W. Patterson for his helpful advice in the biological testing, and to Mr. Soulsby, of the Edinburgh City Abattoir, for the collection of the parasites.

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BY

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SOME OBSERVATIONS ON THE LETHAL EFFECTS OF VARIOUS CHEMICALS AGAINST THE FREE-LIVING STAGES OF SCLEROSTOMES (NEMATODA)*

BY

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After the discovery of the life history of the hookworms of man, control measures included the treatment of night-soil with various readily available chemicals, such as sodium chloride, ammonium sulphate, sodium nitrate, other fertilizers, and with urine to kill the eggs and free-feeding larvae.

In 1933 work was begun at the Institute of Parasitology of McGill University† with a view to finding chemicals which could be used to kill the free-living stages of Sclerostomes in manure heaps. It was soon found that this only dealt with one aspect of the problem, and left the control of larvae in stables and sheds and on pastures unsolved; just as treating night-soil leaves the problem of larvae on the wood and metal of latrines, and faeces in the fields as sources of hookworm infection. For these reasons a much wider range of chemicals was tested. It was also suggested that information on the types of chemicals which are lethal to the free-living stages of bursate nematodes might suggest types of chemicals which would be lethal to some of the nematodes which are parasitic on plants.

In 1946 the work was continued at the Department of Zoology of Edinburgh University.‡ The range of chemicals tested was again considerably widened, and included many which were of only theoretical interest, in order to test some theories which had been formulated on the chemical constitution of substances which are lethal. For this purpose appropriate chemicals were made at the Heriot-Watt College.‡ Furthermore it was hoped that indications might be obtained on the types of chemical which could be used as anthelmintics; especially as anthelmintics against the immature forms of bursate nematodes in the abomasum and small intestine. At present there is no satisfactory anthelmintic against many immature nematodes, a want which constitutes a very serious deficiency in prophylactic dosing of farm animals.

Wright and Schaffer (1932) tested a series of chlorinated aliphatic hydrocarbons against hookworms in dogs and found that anthelmintic effect depended upon solubility in water, length of carbon chain, and the position of the halogen in the molecule. During recent years many chemicals have been tested as anthelmintics in laboratory animals and against plant nematodes. In addition, numerous chemicals

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have been tested *in vitro* by Lamson and Brown (1936) and Baldwin (1943, 1948) with *Ascaris lumbricoides*, by Chance and Mansour (1949) with *Fasciola hepatica*, and by Duguid and Heathcote (1950) with *Moniezia expansa*. These screening tests have given many indications of the types of chemicals which are lethal to various adult helminths. Levine (1949, 1951a and b) screened numerous chemicals against Sclerostome larvae in faeces, from which he was able to draw some conclusions on the types of chemicals which are lethal to the free-living stages of bursate nematodes.

However, at present there is no thoroughly satisfactory anthelmintic for some of the common bursate nematodes of domestic animals; there is no chemical which could be recommended to kill bursate larvae in latrines, stables, sheds, or pens, and there is no practical method of killing larvae on pastures.

The results given in this paper can almost be considered as *in vivo* tests against the free-living stages of bursate nematodes, and, in some respects, as *in vitro* anthelmintic tests against the immature parasitic forms of some genera. The fact that the tests of the chemicals as ovicides and larvicides were in small cultures in a confined space at a comparatively high temperature (25° C.) gave the chemicals greater opportunities of being effective than would occur in practice.

Method

Chemicals have been tested by adding them to 40 g. of horse faeces within five hours of the faeces being passed. When the chemicals were tested in the dry state, they and the faeces were mixed and tied up in butter muslin or cheese cloth, and put into glass preserving jars of about 550 c.c. capacity, fitted with lids without rubber rings. Chemicals in solution or suspension were poured slowly over the faeces which had previously been tied up in butter muslin and placed in the jars. A few chemicals were tested in 80, 16, and 10 g. cultures, contained in proportionately sized jars; the smaller jars were fitted with shives with a small hole bored in them. The cultures were kept for a minimum of ten days, but usually longer, in a constant temperature room, at $25 \pm 2°$ C., which gave the free-living stages more than sufficient time to reach the infective stage. Untreated control cultures were always made. The larvae, if any, were extracted by soaking the cultures in warm water in 8-in. glass funnels. To collect any larvae on the walls of the jars, they were filled with water, which was also poured into the funnels. Two or three days later more warm water was added to the funnels. Six to ten days later the bottom 50 c.c. of each funnel was drawn off into an Erlenmeyer flask. The larvae in this fluid were then counted on a squared glass dish with the help of a dissecting microscope; if the larvae were numerous a dilution technique was used before counting—control cultures usually contained several thousand larvae. This technique has already been described in greater detail by Parnell (1936, 1938).

When a chemical was first tested cultures were made in a wide range of concentrations. After these preliminary cultures had shown the probable quantity of chemical required to kill the free-living stages, cultures were made in duplicate above, at, and below the indicated lethal quantities; and single cultures were made with still greater and lesser amounts of chemical. The final results, therefore, were usually based on three cultures for the quantities of chemical approximating to the lethal percentage, and in addition there were series of one or two cultures with considerably more and considerably less chemical.

The majority of the chemicals were tested by being mixed dry or undiluted in the faeces, and, if soluble in water, were also tested as aqueous solutions; a few chemicals were tested as aqueous suspensions. Amounts up to 20 g. of solid and of 25 c.c. of liquid were

added to 40 g. of faeces; 25 c.c. of fluid is about the maximum quantity that 40 g. of fresh horse faeces will absorb. The more effective the chemicals were the more accurately were their values assessed. When very small amounts of chemical were applied the difference between quantities was only 0.001 g. or 0.001 c.c., but with larger quantities the difference was as much as 4 g. or 5 c.c. Therefore, it follows that with the less lethal chemicals smaller percentages might have been effective. Similarly, if more than three cultures had been made, sometimes the arbitrary 90 and 99.9 reduction percentages might have been slightly different; this is especially true where some larvae avoided the action of the chemical in one culture.

In the following Tables, " very concentrated solutions " implies 1 g. of chemical to 2 or 4 c.c. of water, " concentrated solutions " were 1 g. of chemical to 8 or 20 c.c. of water, " moderately concentrated solutions " were 1 g. of chemical to 50 or 100 c.c. of water, " moderately dilute solutions " were 1 g. of chemical to 200, 300, 400, or 500 c.c. of water, " dilute solutions " were 1 g. of chemical to 600, 800, 1,000, 1,250, and 1,500 c.c. of water, " very dilute solutions " were 1 g. of chemical to 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, and 8,000 c.c. of water, and " extremely dilute solutions " were 1 g. of chemical to 10,000, or more, c.c. of water. The most effective results are quoted for each group of solutions. A few chemicals were also measured as gases.

The description " third stage larvae " has been used in most places in this paper in preference to infective larvae, because it is probable that some of the larvae which reached the third stage in treated cultures, although alive and often active, had been damaged sufficiently by the chemical to reduce considerably their ability to infest a host.

Results

Table I gives the percentages by weight of eighty inorganic chemicals and Table II gives similar figures for nearly two hundred organic chemicals and other substances which gave a 90 and a 99·9 per cent reduction in the number of third stage larvae found in the cultures. Forty-five chemicals and other materials are listed which have no lethal value or such a low value that it was not determined. Reductions in the percentage of larvae recovered caused by alteration in the consistency of the cultures have been ignored, as far as possible. Sometimes insufficient chemical was added to the cultures to enable the lethal value to be determined; this is indicated in Tables I, II, and III by an asterisk (*); where the quantity of solid or undiluted chemical was more or less than 50 per cent it is shown by including the percentage in brackets below the asterisk. When the chemical was in solution the maximum amount applied has been the percentage in 25 c.c. added to 40 g. faeces.

Only the most lethal chemicals were tested as very dilute or extremely dilute solutions; that is, in solutions containing only one gramme of chemical in 2,000 c.c., or more, of water. Table III lists those chemicals which were effective in " very and extremely dilute solutions," and shows the percentages of chemical to faeces required for 90 and 99.9 per cent reductions in the average numbers of third stage larvae recovered.

Although free-feeding larvae are usually more easily killed by unfavourable conditions than are infective larvae, some of the larvae have been able to develop to the third stage before being killed in cultures treated with some chemicals. A few of these chemicals have killed all these larvae, others have only killed some. Chemicals which fairly frequently showed this delayed killing action are listed in Table IV. Often the percentage of chemical necessary to produce this phenomenon varied considerably, depending on whether the chemical was added as a solid or

TABLE I

INORGANIC CHEMICALS

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied — Dry or undiluted	90% — In very concentrated solutions	90% — In concentrated solutions	90% — In moderately concentrated solutions	90% — In moderately dilute solutions	90% — In dilute solutions	99.9% when applied — Dry or undiluted	99.9% — In very concentrated solutions	99.9% — In concentrated solutions	99.9% — In moderately concentrated solutions	99.9% — In moderately dilute solutions	99.9% — In dilute solutions
Sodium compounds:												
Sodium hydroxide	1.9	3.0	1.9	1.25	—	—	2.5	4.0	2.5	—	—	—
Sodium bicarbonate	7.5	—	6.0	—	—	—	20.0	—	7.5	—	—	—
Sodium fluoride	0.5	—	0.9	0.2	0.08	0.06	0.8	—	0.9	0.4	0.25	*
Sodium chloride	2.5	4.1	1.9	*	—	—	5.0	4.1	2.5	*	—	—
Sodium iodide	0.02	0.2	0.12	0.1	0.025	0.02	0.19	0.9	0.37	0.13	0.05	0.04
An aqueous solution of sodium hypochlorite with 1.2% available chlorine	19.0	*	*	—	—	—	25.0	*	*	—	—	—
Sodium chlorate	3.75	4.0	2.5	—	—	—	6.25	5.75	6.0	—	—	—
Sodium sulphate	12.5	14.2	*	—	—	—	20.0	19.0	*	—	—	—
Sodium sulphite	10.0	8.6	*	—	—	—	15.0	25.0	*	—	—	—
Sodium sulphide	10.0	7.2	7.4	—	—	—	12.5	14.4	*	—	—	—
Sodium nitrate	3.7	2.9	2.3	—	—	—	6.2	5.8	3.1	—	—	—
Trisodium phosphate	6.2	7.2	6.0	—	—	—	12.25	12.5	*	—	—	—
Sodium tetraborate	0.63	—	0.9	0.4	—	—	3.75	—	1.8	1.0	—	—
Sodium silicofluoride	1.25	—	—	0.3	0.06	0.06	3.7	—	—	0.5	0.2	*
Potassium compounds:												
Potassium hydroxide	2.5	3.0	2.5	*	—	—	3.75	4.6	4.5	*	—	—
Potassium carbonate	7.5	5.0	4.5	—	—	—	7.5	7.5	7.5	—	—	—
"Muriate of potash," fertilizer	3.7	4.3	3.7	—	—	—	7.5	5.7	6.0	—	—	—
Potassium iodide	0.025	0.6	0.3	0.13	0.04	0.015	0.075	1.2	0.5	0.25	0.06	0.05
Potassium chlorate	15.0	6.2	3.1	*	—	—	35.0	15.5	6.0	*	—	—
Potassium iodate	0.05	—	0.25	0.1	0.05	0.025	0.62	—	0.5	0.19	0.08	0.08
Potassium sulphate	10.0	11.5	7.5	—	—	—	30.0	20.0	*	—	—	—
Potassium nitrate	3.7	4.3	3.0	—	—	—	12.5	4.3	4.5	—	—	—
Potassium cyanate	1.25	1.15	0.9	0.5	*	—	1.9	1.5	1.8	0.75	*	—
162 parts potassium cyanate + 96 parts ammonium carbonate	1.25	1.5	1.25	0.5	—	—	1.9	1.5	1.6	*	—	—
Potassium permanganate	7.5	—	*	—	—	—	25.0	—	*	—	—	—
Ammonia and ammonium compounds:												
Aqueous ammonia (containing 27% NH_3)	1.3	1.5	2.1	*	—	—	1.3	2.1	2.1	*	—	—
Ammonium carbonate	1.9	1.6	1.4	1.0	—	—	2.5	1.9	1.8	*	—	—
Ammonium chloride	1.9	2.1	1.1	1.2	—	—	3.7	4.0	1.1	*	—	—
Ammonium iodide	0.08	0.44	0.19	0.1	0.033	0.024	0.5	1.8	0.5	0.19	0.08	0.09
Ammonium sulphate	5.0	4.3	3.0	—	—	—	10.0	7.2	6.0	—	—	—
Ammonium sulphide (15% aqueous solution)	6.2	4.2	5.5	—	—	—	10.0	6.2	7.0	—	—	—
Ammonium sulphamate	0.82	1.5	0.6	0.5	0.2	—	2.5	2.4	1.85	0.62	*	—
Ammonium nitrate	1.9	3.9	1.9	—	—	—	3.7	4.0	3.0	—	—	—
"Nitro chalk"	6.2	5.0	3.1	—	—	—	7.5	7.2	7.5	—	—	—
"Calnitro"	2.5	4.3	2.5	—	—	—	6.2	7.2	6.0	—	—	—
Diammonium phosphate	3.7	4.3	2.5	—	—	—	6.2	7.2	6.0	—	—	—
Ammonium thiocyanate	0.5	0.8	0.6	0.2	0.13	—	1.9	2.1	1.5	0.5	*	—
Copper compounds:												
Cuprous chloride	3.75	—	—	—	—	—	6.2	—	—	—	—	—
Cupric chloride	1.9	2.5	1.6	0.6	—	—	3.75	3.1	3.1	*	—	—
Cupric sulphate	6.2	4.3	4.5	*	—	—	15.0	8.6	*	*	—	—
Cupric nitrate	3.7	2.5	1.6	1.2	—	—	6.2	4.2	3.1	*	—	—
Magnesium compounds:												
Magnesium chloride	6.2	7.2	6.0	—	—	—	12.5	8.6	7.5	—	—	—
Magnesium sulphate	17.5	15.5	*	—	—	—	35.0	*	*	—	—	—
Kainite (high grade)	3.7	2.9	2.2	—	—	—	5.0	4.0	7.5	—	—	—
Magnesium borate	0.5	—	—	—	—	—	1.9	—	—	—	—	—

TABLE I—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by 90% when applied						99·9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Calcium compounds:												
Bleaching powder	5.0	4.3	3.5	—	—	—	7.5	7.5	5.5	—	—	—
Calcium nitrate	3.7	4.3	3.0	—	—	—	10.0	5.7	7.5	—	—	—
"Calurea"	1.2	0.9	1.2	1.2	—	—	3.7	1.4	1.2	1.2	—	—
16% superphosphate	35.0	—	—	—	—	—	50.0	—	—	—	—	—
20% superphosphate	15.0	—	—	—	—	—	20.0	—	—	—	—	—
Calcium arsenate	1.0	—	—	—	—	—	20.0	—	—	—	—	—
Calcium cyanide	0.05	—	—	—	—	—	0.19	—	—	—	—	—
Calcium cyanamide (powdered)	1.9	1.8	0.6	0.8	—	—	1.9	4.8	1.8	*	—	—
Calcium cyanamide (granular)	1.2	—	—	—	—	—	2.5	—	—	—	—	—
Calcium chloroacetate	1.25	1.15	0.9	0.25	0.25	—	3.75	5.75	1.85	1.0	*	—
Zinc compounds:												
Zinc chloride	1.9	1.6	1.25	0.5	—	—	3.75	4.2	1.9	1.0	—	—
Zinc iodide	0.13	0.35	0.19	0.06	0.02	0.02	0.63	1.0	0.3	0.13	0.05	0.05
Zinc sulphate	1.3	2.5	1.25	0.5	—	—	15.0	5.8	3.1	*	—	—
Zinc sulphide	50.0	—	—	—	—	—	*	—	—	—	—	—
Mercury compounds:												
Mercurous chloride	0.037	—	—	—	—	—	0.1	—	—	—	—	—
Mercuric chloride	0.025	—	0.025	0.008	0.008	0.009	0.062	—	0.1	0.025	0.019	0.017
Ethyl-mercuric chloride	0.004	—	—	—	—	—	0.025	—	—	—	—	—
Ethoxy-ethyl-mercuric chloride	0.025	—	—	—	0.006	0.008	0.37	—	—	—	0.04	0.03
Mercuric iodide	0.12	—	—	—	—	—	3.7	—	—	—	—	—
A seed dressing containing mercury alkoxy-ethyl	1.25	—	—	—	—	—	2.5	—	—	—	—	—
Boron compound:												
Ortho-boric acid	0.63	—	—	—	—	—	1.9	—	—	—	—	—
Carbon compound:												
Carbon disulphide	0.32	—	—	—	—	—	1.6	—	—	—	—	—
Iodine:												
Iodine	0.08	—	—	—	—	—	0.38	—	—	—	—	—
10% colloidal iodine in vegetable oil	0.8	—	—	—	—	—	7.5	—	—	—	—	—
0.12% iodine in oily medium	32.0	—	—	*	—	—	62.0	—	—	*	—	—
"Iodine vermicide"—containing 16% iodine	1.9	—	0.3	0.4	0.08	*	5.0	—	0.08	0.8	0.3	*
Manganese compounds:												
Manganous chloride	2.5	3.1	2.25	1.25	—	—	6.2	6.2	3.0	*	—	—
Manganous sulphate	7.5	4.0	1.5	—	—	—	12.5	8.5	2.5	—	—	—
Iron compounds:												
Ferrous chloride	3.75	2.5	1.85	*	—	—	3.75	5.0	3.1	*	—	—
Ferrous iodide	0.08	—	—	0.12	0.06	0.05	0.8	—	—	0.38	0.1	*
Ferrous sulphate	3.75	4.0	2.5	—	—	—	10.0	7.0	6.0	—	—	—
Ferric chloride	3.75	2.3	2.1	*	—	—	3.75	2.9	3.1	*	—	—
Ferric sulphate	3.7	—	—	—	—	—	5.0	—	—	—	—	—
Cobalt compound:												
Cobalt chloride	3.75	2.9	1.85	0.5	—	—	6.25	5.7	2.45	*	—	—
Nickel compound:												
Nickel chloride	3.75	2.8	1.25	0.5	0.17	—	10.0	5.8	2.5	1.25	0.3	—

TABLE II

ORGANIC CHEMICALS

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by 90% when applied: Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	99.9% when applied: Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
ALIPHATIC COMPOUNDS												
Paraffins:												
44.3% Naphtha + 5.3% oleic acid + 1.5% Teepol X + 0.6% NaOH and water ..	0.23	—	0.2	0.23	0.23	—	0.35	—	0.44	0.34	0.29	—
Halogen derivatives of paraffins:												
Methyl bromide	0.009	—	—	—	—	—	0.02	—	—	—	—	—
Methyl iodide	0.006	—	—	0.006	0.004	0.003	0.012	—	—	0.012	0.007	0.007
Ethyl chloride	4.5	—	—	—	*	—	*(57.5)	—	—	—	—	*
Ethyl bromide	0.15	—	—	—	—	—	0.4	—	—	—	—	—
Ethyl iodide	0.02	—	—	—	0.024	0.015	0.07	—	—	—	0.035	0.04
n-Propyl bromide	0.11	—	—	—	*	—	0.25	—	—	—	*	—
n-Propyl iodide	0.033	—	—	—	—	0.03	0.066	—	—	—	—	0.09
n-Butyl chloride	0.74	—	—	—	—	—	1.12	—	—	—	—	—
n-Butyl bromide	0.1	—	—	—	—	—	0.32	—	—	—	—	—
tert.-Butyl bromide	1.2	—	—	—	—	—	1.5	—	—	—	—	—
n-Amyl bromide	0.3	—	—	—	—	—	0.6	—	—	—	—	—
n-Hexyl bromide	0.072	—	—	—	—	—	0.16	—	—	—	—	—
n-Heptyl bromide	0.043	—	—	—	—	—	0.14	—	—	—	—	—
n-Octyl bromide	0.055	—	—	—	—	—	0.14	—	—	—	—	—
Methylene dichloride	0.67	—	—	—	—	—	2.5	—	—	*	—	—
Methylene dibromide	0.47	—	—	0.77	0.5	—	0.63	—	—	1.2	0.8	—
Methylene diiodide	0.008	—	—	0.04	0.012	0.004	0.02	—	—	0.23	0.07	0.03
Ethylene dichloride	0.5	—	—	*	*	—	1.0	—	—	*	*	—
Ethylidene dichloride	1.17	—	—	—	*	—	2.2	—	—	—	*	—
10% methyl bromide + 67.5% ethylene dichloride, 22.5% carbon tetrachloride	0.47	—	—	—	—	—	0.78	—	—	—	—	—
Ethylene dibromide	0.08	—	—	—	—	—	0.08	—	—	—	—	—
1 : 3-Dichloropropylene + 1 : 2-Dichloropropane + higher chlorides (D.D. mixture) ..	0.01	—	—	—	—	—	0.019	—	—	—	—	—
Dichloropentanes	0.27	—	—	—	—	—	0.54	—	—	—	—	—
Chloroform	1.2	—	—	—	*	—	3.75	—	—	—	*	—
Bromoform	0.18	—	—	—	0.22	0.15	0.29	—	—	—	0.36	0.22
Iodoform	0.075	—	—	—	—	—	0.18	—	—	—	—	—
Chloropicrin	0.02	—	—	—	—	—	0.02	—	—	—	—	—
1 : 1 : 1-Trichloroethane	1.1	—	—	—	—	—	2.5	—	—	—	—	—
Carbon tetrachloride	1.3	—	—	—	—	—	5.9	—	—	—	—	—
Carbon tetrabromide	0.019	—	—	—	—	—	0.025	—	—	—	—	—
1 : 1 : 2 : 2-Tetrachloroethane ..	0.1	—	—	—	—	—	0.13	—	—	—	—	—
Pentachloroethane	0.21	—	—	—	—	—	0.42	—	—	—	—	—
Hexachloroethane	0.12	—	—	—	—	—	0.25	—	—	—	—	—
Halogen derivatives of ethylene:												
Dichlorethylene (*cis*)	0.65	—	—	—	—	—	1.06	—	—	—	—	—
Dichlorethylene (*trans*)	0.62	—	—	—	—	—	1.56	—	—	—	—	—
Trichlorethylene	0.36	—	—	—	—	*	0.72	—	—	—	—	*
Perchloroethylene	0.31	—	—	—	—	—	0.31	—	—	—	—	—
Alcohols:												
Methyl alcohol	4.0	2.65	3.4	—	—	—	5.0	6.0	*	—	—	—
Ethyl alcohol	3.0	2.0	2.8	—	—	—	5.0	3.3	4.4	—	—	—
n-Propyl alcohol	1.5	1.3	1.7	*	—	—	1.5	1.6	2.2	*	—	—
iso-Propyl alcohol	1.5	1.6	1.6	*	—	—	3.0	2.0	2.3	*	—	—
n-Butyl alcohol	0.66	—	0.95	0.8	—	—	1.5	—	1.7	1.0	—	—
iso-Butyl alcohol	0.8	—	0.9	0.97	—	—	3.0	—	1.1	*	—	—
tert.-Butyl alcohol	1.5	1.0	1.1	*	—	—	1.5	1.3	1.6	*	—	—
Ethers:												
Ether	1.8	—	1.3	—	—	—	3.6	—	*	—	—	—
Dichlorodiethyl ether	0.06	—	—	0.05	0.05	0.05	0.1	—	—	0.09	0.11	0.11
50% β-butoxy-β-thiocyanodiethyl ether + 50% petroleum distillate of the kerosene type	0.46	—	—	—	—	—	0.75	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied: Dry or undiluted	90% when applied: In very concentrated solutions	90% when applied: In concentrated solutions	90% when applied: In moderately concentrated solutions	90% when applied: In moderately dilute solutions	90% when applied: In dilute solutions	99.9% when applied: Dry or undiluted	99.9% when applied: In very concentrated solutions	99.9% when applied: In concentrated solutions	99.9% when applied: In moderately concentrated solutions	99.9% when applied: In moderately dilute solutions	99.9% when applied: In dilute solutions
Sulphides:												
Tetramethyl thiuram monosulphide	1.9	—	0.9	0.25	0.19	—	40.0	—	1.9	1.25	*	—
46% tetramethyl thiuram disulphide	5.0	—	0.9	0.2	0.06	0.1	7.5	—	1.8	0.6	0.16	*
Formaldehyde and derivative:												
Formalin	2.0	0.7	0.5	0.27	0.2	—	2.0	1.4	1.2	0.55	0.35	—
Hexamethylene tetramine	7.5	4.5	*	—	—	—	12.5	18.0	*	—	—	—
Ketone:												
Acetone	3.6	3.3	4.4	—	—	—	8.0	6.6	5.5	—	—	—
Soaps:												
Hard soap (Castile)	—	11.0	—	—	—	—	—	*	—	—	—	—
Soft soap	—	*	—	—	—	—	—	*	—	—	—	—
Esters:												
Ethyl iodoacetate	0.06	—	—	—	—	—	0.19	—	—	—	—	—
Dodecyl thiocyanate	3.75	—	—	—	—	—	60.0	—	—	—	—	—
50% mixed thiocyanates + 50% petroleum distillate of the kerosene type	0.46	—	—	—	—	—	0.75	—	—	—	—	—
Hexaethyl tetraphosphate	3.8	—	—	*	—	—	6.2	—	—	*	—	—
Di-ethyl-*p*-nitrophenylthiophosphate	0.8	—	—	—	—	—	39.0	—	—	—	—	—
Sodium alkyl sulphates (Teepol X)	10.5	—	1.6	*	—	—	66.0	—	*	*	—	—
Amines:												
25% dimethylamine	3.5	1.9	2.7	*	—	—	3.5	3.1	5.2	*	—	—
Cetyltrimethylammonium bromide	3.75	—	1.8	0.75	—	—	30.0	—	*	*	—	—
20% solution of alkyl ammonium bromides	—	—	1.5	—	—	—	—	—	*	—	—	—
10% alkyl-dimethyl-benzyl ammonium chlorides	25.0	6.2	2.4	0.6	—	—	37.5	*	*	*	—	—
Allyl compounds:												
Allyl isothiocyanate	0.0015	—	—	—	0.002	0.002	0.0025	—	—	—	0.004	0.003
Allyl chloride	0.1	—	—	—	—	—	0.23	—	—	—	—	—
Allyl bromide	0.007	—	—	—	—	—	0.026	—	—	—	—	—
Allyl iodide	0.0012	—	—	—	—	—	0.003	—	—	—	—	—
Allyl alcohol	0.05	0.05	0.05	0.03	0.05	0.07	0.16	0.08	0.1	0.08	0.11	*
Dihydric alcohol and derivative:												
2-Ethyl-hexanediol-1 : 3	10.0	—	—	—	—	—	19.0	—	—	—	—	—
"Lubrol W."	—	—	1.0	1.0	—	—	—	—	*	*	—	—
Keto-acid:												
Pyruvic acid	6.3	3.1	1.9	1.55	—	—	15.7	5.3	3.0	*	—	—
Carbonic acid derivatives:												
80% zinc dimethyldithiocarbamate	*	—	1.5	0.4	0.16	—	*	—	3.1	0.6	*	—
80% ferric dimethyldithiocarbamate	1.25	—	0.9	0.4	0.2	—	*	—	2.4	0.6	*	—
Urea	0.8	0.7	0.6	0.5	—	—	0.8	0.7	0.9	0.6	—	—
Guanidine hydrochloride	—	—	0.9	0.3	—	—	—	—	1.5	0.6	—	—
Methylguanidine sulphate	—	—	0.9	0.5	—	—	—	—	1.8	1.2	—	—
as-Dimethylguanidine sulphate	—	—	0.9	0.5	—	—	—	—	1.5	1.0	—	—
Diphenylguanidine	—	—	2.5	0.6	—	—	—	—	*	*	—	—
Creatine	—	—	—	0.9	—	—	—	—	—	*	—	—
Creatinine	—	—	0.9	1.0	—	—	—	—	*	1.2	—	—
Thiourea	0.1	—	0.3	0.1	0.06	0.06	0.4	—	1.8	0.3	0.2	*
Potassium xanthogenate	0.5	0.7	0.9	0.2	0.16	—	1.9	2.4	1.2	0.6	*	—
Esters of dibasic acids:												
Butyl mesityl oxalic ester	10.4	—	—	—	—	—	13.0	—	—	—	—	—
Butyl mesityl oxide oxalate	19.0	—	—	—	—	—	38.0	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by: 90% when applied: Dry or undiluted	90%: In very concentrated solutions	90%: In concentrated solutions	90%: In moderately concentrated solutions	90%: In moderately dilute solutions	90%: In dilute solutions	99.9% when applied: Dry or undiluted	99.9%: In very concentrated solutions	99.9%: In concentrated solutions	99.9%: In moderately concentrated solutions	99.9%: In moderately dilute solutions	99.9%: In dilute solutions
Ureides and purine derivatives:												
Barbituric acid	3.75	—	—	1.25	*	—	17.5	—	—	*	*	—
Allantoin	1.25	—	—	—	—	—	*	—	—	—	—	—
Xanthine	7.5	—	—	—	*	—	*	—	—	—	*	—
Guanine	10.0	—	—	—	—	—	(10.0) * (15.0)	—	—	—	—	—
CARBOCYCLIC COMPOUNDS												
Hydrocarbons:												
Benzene	0.56	—	—	—	—	—	0.72	—	—	—	—	—
Toluene	0.16	—	—	—	—	—	0.86	—	—	—	—	—
Xylenes	0.09	—	—	—	—	—	0.16	—	—	—	—	—
p-Cymene	0.09	—	—	—	—	—	0.16	—	—	—	—	—
Halogen derivatives:												
Chlorobenzene	0.11	—	—	—	—	—	0.2	—	—	—	—	—
Bromobenzene	0.09	—	—	—	—	—	0.12	—	—	—	—	—
Iodobenzene	0.11	—	—	—	—	—	0.18	—	—	—	—	—
o-Dichlorobenzene	0.11	—	—	—	—	—	0.24	—	—	—	—	—
p-Dichlorobenzene	0.12	—	—	—	—	—	0.37	—	—	—	—	—
2:3:5:6-Tetrachloronitrobenzene	17.5	—	—	—	—	—	*	—	—	—	—	—
Cyclohexyl bromide	0.16	—	—	—	—	—	0.32	—	—	—	—	—
Mercury compounds:												
Phenyl mercuri-acetate	0.01	—	—	—	—	—	0.12	—	—	—	—	—
An organic mercurial seed dressing containing 1% mercury	0.8	—	—	—	—	—	3.75	—	—	—	—	—
Amino-compounds:												
Aniline	0.04	—	—	0.06	0.05	0.03	0.12	—	—	0.13	0.13	0.13
p-Aminophenyl arsonic acid	1.25	—	—	0.6	—	—	2.5	—	—	*	—	—
Diphenylamine	12.5	—	—	—	—	*	*	—	—	—	—	*
Diphenylamine chloroarsine	10.0	—	—	—	—	—	20.0	—	—	—	—	—
Azo-compounds:												
Azobenzene	1.25	—	—	—	—	—	2.5	—	—	—	—	—
Amino-azobenzene-hydrochloride	10.0	—	—	—	—	—	*	—	—	—	—	—
Phenols:												
Phenol	0.4	0.3	0.3	0.2	0.11	0.11	1.3	1.9	0.5	0.3	0.3	*
40% low boiling phenols in emulsion	0.8	1.0	0.4	0.3	0.3	—	2.6	5.1	0.9	1.0	*	—
40% high boiling phenols in emulsion	5.0	2.5	1.2	0.5	—	—	12.5	5.0	1.5	1.2	—	—
60% high boiling phenolic bodies in emulsion	18.75	3.75	1.8	0.75	—	—	31.0	10.0	2.4	*	—	—
Sodium 2:4:5-trichlorophenate	0.5	2.0	0.9	0.1	0.06	—	1.25	4.3	1.6	0.5	*	—
o-Iodo-phenol	—	—	—	0.3	0.1	—	—	—	—	0.5	0.3	—
o-Nitro-phenol	0.25	—	—	—	—	—	0.25	—	—	—	—	—
p-Nitro-phenol	1.25	—	—	0.13	0.1	—	5.0	—	—	0.6	0.25	—
Sodium *o*-phenyl phenate	5.0	5.25	0.5	0.25	0.1	—	10.0	6.6	3.0	0.6	0.25	—
Sodium *m*-chloro-*p*-phenyl-phenate	10.0	7.2	1.5	0.25	0.13	—	17.5	14.3	*	0.6	*	—
2-Cyclohexyl cyclohexanol+2-phenyl cyclohexanol	4.7	—	—	—	—	—	7.1	—	—	—	—	—
50% cresol	3.7	1.0	0.9	0.4	0.2	—	7.5	2.5	1.5	0.6	*	—
Monochloroxylenols with some dichloroxylenols	0.5	—	—	—	—	—	3.8	—	—	—	—	—
2:4-Dichloro-*sym.*-xylenol	1.9	—	—	—	—	—	20.0	—	—	—	—	—
"Dettol" (halogenated xylenol in aromatic oils)	1.0	1.25	0.6	0.5	—	—	2.5	3.3	1.8	1.0	—	—
Thymol	1.0	—	—	—	—	—	1.9	—	—	—	—	—
Resorcinol	0.25	1.0	0.6	0.13	0.08	0.09	1.9	5.0	1.5	1.0	*	*
4-*n*-Hexylresorcinol	6.2	—	—	—	—	—	*	—	—	—	—	—
Ketone:												
p-Chloro-acetophenone	1.25	—	—	—	—	—	2.5	—	—	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by											
	90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Monobasic acids and derivatives:												
Benzoic acid	17.5	—	—	—	*	—	*	—	—	—	*	—
Benzyl benzoate	28.0	—	—	—	—	—	* (70.0)	—	—	—	—	—
Chloromethylphenoxy-acetic acid	18.7	—	*	*	—	—	31.0	—	*	*	—	—
1% trichlorophenylmethyliodo-salicylate	19.0	8.3	7.0	—	—	—	25.0	16.6	*	—	—	—
Ester of dibasic acid:												
Dimethyl phthalate	11.9	—	—	—	—	—	45.0	—	—	—	—	—
Triphenylmethane dyes:												
Malachite green	3.75	—	—	1.0	—	—	12.5	—	—	*	—	—
Brilliant green	7.5	—	3.1	1.25	—	—	*	—	*	*	—	—
Magenta	50.0	—	*	*	—	—	*	—	*	*	—	—
Methyl violet	30.0	—	—	*	—	—	*	—	—	*	—	—
Gentian violet	35.0	—	3.1	1.25	—	—	*	—	*	*	—	—
Methyl green	7.5	—	2.5	1.0	—	—	17.5	—	*	*	—	—
Terpenes:												
Turpentine	0.55	—	—	—	—	—	1.3	—	—	—	—	—
Essential oil ex *Artemisia maritima* containing 65% β-thujone and 16% 1:8-cineole	0.1	—	—	—	—	—	0.17	—	—	—	—	—
10% of essential oil of *Artemisia maritima* in an aqueous alcoholic soap basis	0.74	0.75	0.75	0.5	*	—	0.91	1.1	1.3	1.1	*	—
β-Thujone	0.092	—	—	—	—	—	0.092	—	—	—	—	—
1:8-Cineole	0.23	—	—	—	—	—	0.58	—	—	—	—	—
Oil of chenopodium	0.1	—	—	—	—	—	0.25	—	—	—	—	—
5% oil of chenopodium with 95% castor oil	2.5	—	—	—	—	—	6.2	—	—	—	—	
Naphthalene and derivative:												
Naphthalene	0.19	—	—	—	—	—	0.38	—	—	—	—	—
β-Naphthol	12.5	—	—	—	—	—	*	—	—	—	—	—
Saponins:												
Saponin mixture	20.0	25.0	6.0	—	—	—	*	*	*	—	—	—
HETEROCYCLIC COMPOUNDS												
Benzthiazole:												
2-Mercaptobenzthiazole	35.0	—	—	—	—	—	*	—	—	—	—	—
Pyridine and derivatives:												
Pyridine	0.19	0.16	0.18	0.19	0.19	—	0.25	0.25	0.24	0.25	0.25	—
α-Picoline	0.09	0.07	0.085	0.07	0.09	0.1	0.17	0.12	0.17	0.14	0.12	*
β-Picoline	0.18	0.12	0.11	0.14	0.15	—	0.18	0.16	0.17	0.23	0.23	—
γ-Picoline	0.18	0.12	0.11	0.14	0.12	—	0.18	0.16	0.17	0.23	0.24	—
2:6-Lutidine	0.09	0.07	0.05	0.07	0.08	0.08	0.17	0.12	0.08	0.09	0.12	*
32.9% β-Picoline+16.9% γ-picoline+50.2% 2:6-lutidine	0.23	0.12	0.1	0.12	0.12	—	0.23	0.15	0.13	0.23	0.15	—
2:4-Lutidine+2:5-lutidine	0.1	—	0.065	0.07	0.06	0.06	0.18	—	0.12	0.14	0.1	0.1
2:4:6-Collidine	0.17	—	—	0.11	0.06	0.09	0.23	—	—	0.17	0.12	*
3:5-Lutidine+2:3:6-collidine+2:4:6-collidine	0.12	—	—	0.09	0.05	0.05	0.24	—	—	0.19	0.15	0.08
2:4:5-Collidine+o-toluidine+p-toluidine	0.16	—	—	0.11	0.06	0.05	0.34	—	—	0.17	0.11	0.1
Quinoline derivatives:												
Quinoline	0.54	—	0.52	0.27	0.09	0.11	6.9	—	1.95	0.7	0.18	*
8-Hydroxyquinoline sulphate	0.19	0.7	0.3	0.2	0.06	0.04	0.5	1.3	0.9	0.3	0.1	0.07
Acridine derivative:												
Acriflavine	2.5	—	2.5	—	—	—	6.25	—	5.6	—	—	—

TABLE II—*continued*

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by											
	90% when applied						99.9% when applied					
	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions	Dry or undiluted	In very concentrated solutions	In concentrated solutions	In moderately concentrated solutions	In moderately dilute solutions	In dilute solutions
Benzthiazine derivatives:												
3-Keto-2 : 3-dihydro-benz-1 : 4-thiazine	5.0	—	—	0·5	—	—	25.0	—	—	*	—	—
3-Keto-2: 3-dihydro-6: 7-dimethoxy-benz-1: 4-thiazine	50.0	—	—	*	—	—	*	—	—	*	—	—
Hydrochloride of 6-amino 3-keto-2: 3-dihydro-benz-1: 4-thiazine	7.5	—	—	*	—	—	25.0	—	—	*	—	—
Phenoxthine:												
Phenoxthine	1.25	—	—	—	—	*	*	—	—	—	—	*
Phenothiazine and derivatives:												
Phenothiazine	50.0	—	—	—	—	0.04	*	—	—	—	—	*
Mercurated phenothiazine	0.019	—	—	0.06	0.04	0.05	0.38	—	—	0.6	*	*
3: 7-Dinitro-10-acetyl-phenothiazine-5-sulphoxide	50.0	—	*	—	—	—	*	—	*	—	—	—
Phenothiazine sulphoxide	*	—	—	0.4	—	—	*	—	—	*	—	—
Thionol hydrochloride	35.0	—	—	—	—	*	40.0	—	—	*	—	*
Phenothiazone	40.0	—	—	0.5	0.31	0.05	*	—	—	*	*	*
Lauth's violet	3.75	—	—	*	—	—	12.5	—	—	*	—	—
Lauth's violet $\rightleftharpoons$ (phenol)$_2$	2.5	—	—	*	—	—	5.0	—	—	*	—	—
Lauth's violet $\rightleftharpoons$ (β-naphthol)$_2$	5.0	—	—	*	—	—	*	—	—	*	—	—
Lauth's violet $\rightleftharpoons$ (salicylic acid)$_2$	17.5	—	—	*	—	—	35.0	—	—	*	—	—
Lauth's violet $\rightleftharpoons$ (2 - hydroxy - 3-naphthoic acid)$_2$	30.0	—	—	*	—	—	*	—	—	*	—	—
Lauth's violet $\rightleftharpoons$ (1-phenyl-3-methyl-5-pyrazolone)$_2$	50.0	—	—	*	—	—	*	—	—	*	—	—
Brilliant alizarin	25.0	—	*	—	—	—	*	—	*	—	—	—
Methylene blue	50.0	12.0	2.5	—	—	—	*	*	*	—	—	—
Alkaloids:												
Amphetamine sulphate	—	—	—	0.38	0.13	—	—	—	0.75	*	—	—
40% nicotine sulphate	5.7	1.7	0.4	0.28	0.28	—	11.4	7.2	2.7	*	*	—
Tobacco dust	10.0	—	—	—	—	—	*	—	—	—	—	—
Arecoline hydrobromide	0.25	—	—	0.06	—	—	1.25	—	—	0.25	—	—
Quinine acid hydrochloride	6.25	—	*	*	—	—	17.5	—	*	*	—	—
Emetine hydrochloride	2.5	—	—	0.4	—	—	*	—	—	*	—	—
							(10.0)					
Plant product:												
Crushed flax seed	30.0	—	—	—	—	—	62.5	—	—	—	—	—
Urines and manure:												
Fresh poultry manure	40.0	—	—	—	—	—	50.0	—	—	—	—	—
Fresh horse urine	19.0	17.0	—	—	—	—	25.0	31.0	—	—	—	—
Fresh cow urine	37.5	—	—	—	—	—	60.0	—	—	—	—	—
Fresh human urine	50.0	*	—	—	—	—	*	*	—	—	—	—
Fresh dog urine	12.5	9.4	—	—	—	—	50.0	12.5	—	—	—	—

undiluted, or in strong or weak solutions. Frequently there were considerable differences in the minimum percentages of chemical required to produce this phenomenon even between solutions of the concentrated group or between solutions of the dilute group. The minimum percentages of chemical which had this effect, when applied as a solid or undiluted, or in concentrated or in dilute solutions, are shown in Table IV. Some chemicals had this effect in all or practically all the cultures; other chemicals only had this effect in some of the cultures; when this effect occurred fairly regularly the percentages in Table IV have been printed in italic type. Undoubtedly the list would have been longer if many of the chemicals had not already killed the larvae in the earlier stages. Many of these chemicals

TABLE III

The chemicals are listed which in 1 : 2,000 or in weaker solutions reduced the numbers of larvae by 90 per cent and 99.9 per cent; the percentages of chemical required to do this are also given

Chemical	Percentage of chemical to fresh faeces required to reduce the number of larvae by			
	90% when applied		99.9% when applied	
	In very dilute solutions	In extremely dilute solutions	In very dilute solutions	In extremely dilute solutions
Sodium iodide	0.018	—	*	—
Potassium iodide	0.016	—	*	—
Potassium iodate	0.025	—	*	—
Zinc iodide	0.016	—	*	—
Mercuric chloride	0.01	—	0.017	—
Ethoxy-ethyl-mercuric chloride	0.008	—	0.017	—
Iodine	0.02	—	*	—
Methyl iodide	0.003	0.002	0.009	0.007
Ethyl iodide	0.015	—	0.04	—
n-Propyl iodide	0.044	—	*	—
Methylene iodide	0.005	0.005	0.02	0.02
Iodoform	—	0.006	—	*
Allyl isothiocyanate	0.001	0.003	0.002	*
Phenothiazine	0.03	—	*	—

might not have killed the third stage larvae if they had not been able to damage the developing larvae.

Dilute solutions of sodium 2: 4: 5-trichlorophenate were the only dilute solutions which sometimes allowed some of the larvae to develop to the third stage before all the larvae died: the minimum concentration of this compound which did this was 0.3 g./100 c.c.

A few chemicals kill the eggs. Although this technique does not lend itself to the recovery of dead eggs, they were seen in a few of the cultures treated with potassium cyanate, 25 per cent (w/v) aqueous dimethylamine, diphenylamine, high boiling phenols, sodium 2: 4: 5-trichlorophenate, *o*-nitrophenol, sodium *o*-phenyl phenate, and sodium *m*-chloro-*p*-phenyl phenate. Less frequently dead eggs were seen in a few of the cultures treated with thiocyanate mixtures, a few of the halogen derivatives of paraffins, ethers, phenols and pyridine derivatives, and still less frequently a few eggs were seen in cultures treated with other chemicals.

A few chemicals occasionally make some of the larvae exsheath after they have reached the third stage; this was most noticeable with sodium hydroxide, sodium bicarbonate, aqueous sodium hypochlorite (1.2 per cent available chlorine), potassium hydroxide, and potassium permanganate.

Some chemicals stain the larvae and occasionally the stained larvae remain active. Chemicals which most frequently stain or darken larvae include calcium chloroacetate, ferric chloride, aminoazobenzene hydrochloride, malachite green, brilliant green, magenta, methyl violet, gentian violet, methyl green, acriflavine, phenoxthine, phenothiazone, Lauth's violet $=(\beta\text{-naphthol})_2$, and methylene blue.

Occasionally *Trichostrongylus axei* larvae have been seen in these cultures; they and some of the larger larvae seem to be more resistant to some chemicals than are the smaller species of Sclerostomes.

Many chemicals and plant products have been tested which have little or no lethal effect on either the eggs or the free-living larvae. Dibutyl phthalate at 65 per cent, crushed sorgeum grass at 62.5 per cent, crushed sudan grass at 62.5 per cent, crushed tulip tree leaves at 62.5 per cent, Velsicol 1068 at 60 per cent did not produce a 90 per cent reduction in the number of larvae. The addition of 62.5 per cent of

TABLE IV

The chemicals which often allow some of the larvae to develop and then kill some or all of them are listed, with the minimum percentages of chemical which did this when applied as a solid or undiluted and in concentrated and dilute solutions. When there were dead third stage larvae in all or practically all the cultures the percentages are printed in italic type

Chemical	Minimum percentages of chemical which sometimes allow larvae to develop and then kill				
	Some			All	
	Chemical as a solid or undiluted	Chemical in concentrated solutions	Chemical in dilute solutions	Chemical as a solid or undiluted	Chemical in concentrated solutions
Sodium hydroxide	0.8	0.9	—	—	—
Sodium bicarbonate	7.5	—	—	—	—
Sodium iodide	—	0.04	0.03	—	—
Sodium tetraborate	0.63	—	—	—	—
Sodium silicofluoride	*0.25*	0.1	0.1	*0.8*	—
Potassium hydroxide	0.8	1.2	—	—	—
Potassium chlorate	5.0	1.0	—	—	—
Potassium permanganate	*1.9*	—	—	15.0	—
Ammonium thiocyanate	*0.25*	*0.2*	*0.2*	—	—
Cuprous chloride	*0.8*	—	—	*5.0*	—
Cupric chloride	*1.9*	*0.8*	—	—	1.25
Cupric sulphate	*1.9*	*1.0*	—	—	—
Cupric nitrate	*1.9*	*0.5*	—	*2.5*	*2.2*
Magnesium borate	0.38	—	—	—	—
Calcium oxide	20.0	—	—	50.0	—
Calcium hydroxide	50.0	—	—	—	—
Calurea	1.2	1.1	—	—	—
16% superphosphate	20.0	—	—	—	—
Calcium arsenate	*1.25*	—	—	—	—
Calcium chloroacetate	1.25	0.6	—	—	—
Zinc chloride	*1.25*	*0.5*	—	—	2.6
Zinc sulphate	*1.9*	*0.6*	—	—	—
Zinc sulphide	30.0	—	—	—	—
Ethyl-mercuric chloride	0.008	—	—	—	—
Ethoxy-ethyl-mercuric chloride	*0.02*	—	0.02	0.25	—
Mercuric iodide	*0.05*	—	—	1.0	—
Ferrous chloride	—	1.4	—	—	2.9
Ferrous sulphate	1.9	2.4	—	—	—
Ferric sulphate	2.5	—	—	—	—
Nickel chloride	2.5	1.7	0.1	—	—
Iodoform	0.05	—	—	—	—
Dichlorodiethyl ether	0.06	0.06	0.04	—	—
Tetramethylthiuram monosulphide	0.6	0.25	0.16	*3.75*	—
46% tetramethylthiuram disulphide	5.0	0.4	0.1	—	—
Hexamethylene tetramine	3.75	1.8	—	—	—
50% mixed thiocyanates	0.46	—	—	—	—
Hexaethyl tetraphosphate	*2.5*	—	—	—	—
Di-ethyl-*p*-nitrophenyl-thiophosphate	*0.32*	—	—	*4.7*	—
Sodium alkyl sulphates (Teepol X)	*5.25*	1.0	—	52.5	—
25% dimethylamine	2.3	1.7	—	—	—
Cetyltrimethylammonium bromide	*3.75*	1.1	—	—	—
20% solution of alkylammonium bromides	—	2.4	—	—	—
80% zinc dimethyl dithiocarbamate	*0.5*	0.4	0.2	—	—
80% ferric dimethyl dithiocarbamate	*0.6*	0.25	0.2	—	—
Guanidine hydrochloride	—	0.3	—	—	—
Methylguanidine sulphate	—	0.5	—	—	—
as-Dimethylguanidine sulphate	—	0.25	—	—	—
Diphenylguanidine	—	0.37	—	—	—
Creatine	—	0.5	—	—	—
Creatinine	—	0.9	—	—	1.85
Thiourea	0.19	0.1	0.06	—	—
Allantoin	1.0	—	—	*1.25*	—
Xanthine	*1.25*	—	—	10.0	—
Guanine	*1.0*	—	—	—	—
2:3:5:6-Tetrachloronitrobenzene	*1.9*	—	—	—	—
Benzene hexachloride	3.75	—	—	—	—
Phenyl mercuri-acetate	0.025	—	—	—	—
An organic mercurial seed dressing, containing 1% mercury	0.82	—	—	2.5	—
Aniline	0.01	—	—	—	—
Diphenylamine	*0.25*	—	—	—	—
Diphenylamine chloroarsine	6.25	—	—	—	—
Azobenzene	*0.6*	—	—	—	—
Aminoazobenzene hydrochloride	*0.6*	—	—	—	—
40% high boiling phenols in emulsion	5.0	2.0	—	—	—
Sodium 2:4:5-trichlorophenate	*0.12*	*0.03*	*0.03*	*0.8*	*0.6*
o-Iodophenol	—	0.19	0.19	—	—
p-Nitrophenol	*0.25*	0.12	—	*1.25*	—

TABLE IV—*continued*

Chemical	Minimum percentages of chemical which sometimes allow larvae to develop and then kill				
	Some			All	
	Chemical as a solid or undiluted	Chemical in concentrated solutions	Chemical in dilute solutions	Chemical as a solid or undiluted	Chemical in concentrated solutions
Sodium-*o*-phenyl phenate	*1.9*	0.3	—	*7.5*	4.1
Sodium *m*-chloro-*p*-phenyl phenate	*1.25*	*0.3*	0.2	*12.5*	*6.0*
2-Cyclohexyl cyclohexanol + 2-phenyl cyclohexanol	*1.8*	—	—	—	—
50% cresol	5.0	—	—	6.25	—
Monochloroxylenols with some dichloroxylenols	*0.2*	—	—	1.9	—
2 : 4-Dichloro-sym.-xylenol	*0.5*	—	—	*2.5*	—
4-Hexyl resorcinol	*2.5*	—	—	*7.5*	—
3 : 4-Methylenedioxy-phenyl-*n*-propyl-benzyl sulphide	2.5	—	—	—	—
p-Chloroacetophenone	*0.5*	—	—	1.25	—
Benzoic acid	*5.0*	—	—	*30.0*	—
Benzyl benzoate	*4.0*	—	—	—	—
Dimethyl phthalate	*4.5*	—	—	—	—
Malachite green	*5.0*	—	—	—	—
Brilliant green	*3.75*	—	—	—	—
Methyl violet	*7.5*	—	—	—	—
Gentian violet	*1.9*	3.1	—	—	—
Methyl green	*5.0*	—	—	—	—
β-Naphthol	*1.25*	—	—	*5.0*	—
2-Mercapto-benzthiazole	*2.5*	—	—	—	—
Quinoline	*0.27*	—	—	2.7	—
8-Hydroxyquinoline sulphate	0.19	*0.2*	0.1	—	0.8
6-Amino-3-keto-2 : 3-dihydrobenz-1 : 4-thiazine hydrochloride	5.0	—	—	—	—
Phenoxthine	*0.25*	—	0.03	—	—
Phenothiazone	*2.5*	0.5	0.06	—	—
Lauth's violet = ≥(β-naphthol)$_2$	*3.75*	0.4	—	—	—
Lauth's violet = ≥(2-hydroxy-3-naphthoic acid)$_2$	12.5	—	—	—	—
Methylene blue	30.0	—	—	—	—
Crushed sorgeum grass	50.0	—	—	—	—
Crushed sudan grass	62.5	—	—	—	—
Crushed flax seed	20.0	—	—	—	—
Fresh poultry manure	*20.0*	—	—	40.0	—

calcium oxide, of calcium hydroxide, of ground limestone, of basic slag, or of calcium phosphate (as rock phosphate) did not cause a 90 per cent reduction in the numbers of the larvae; but 20 per cent, and sometimes less, of calcium oxide and 50 per cent of calcium hydroxide killed some of the larvae after they had reached the infective stage, and 50 per cent of the former killed them all.

A 90 per cent reduction in the number of larvae was not caused by the addition to 40 g. of fresh horse faeces of 20 g. of the following substances: sublimed sulphur, ferrous sulphide, ferric oxide, cystine (but sometimes 15 per cent, or more, killed some larvae after they had developed), benzene hexachloride and its *alpha*, *beta*, *gamma*, *and delta* isomers separately (however, in some cultures treated with benzene hexachloride many of the larvae died after they had developed to the third stage), 1 : 1 : 1-trichloro-2 : 2-di-*p*-chlorophenylethane (DDT), (the addition to a 44.3 per cent naphtha emulsion of 12.66 per cent of DDT, of DDD, of benzene hexachloride, or of its four isomers did not increase the lethal values of the emulsion), salicylanilide, santonin, derris powder, 6-amino-3-keto-2 : 3-dihydro-benz-1 : 4-thiazine, 6-chloro-3-keto-2 : 3-dihydro-benz-1 : 4-thiazine, 10-acetyl-phenothiazine, thionol, powdered areca nut, white hellebore powder, powdered cousso, pyrethrum powder, mowrah meal, and yeast; however, a few of these substances did kill some of the larvae after they had developed. Although 50 per cent of *m*-methoxy-*p*-hydroxy-benzaldehyde (vanillin) did not produce a 90 per cent reduction in the numbers of larvae, even small quantities caused some reduction.

The addition of 37 per cent of a 25 per cent emulsion of Filix mas reduced the number of larvae by 90 per cent, but even the addition of nearly twice as much did not give a 99.9 per cent kill; therefore much of the reduction in numbers may have been caused by the alteration in the physical condition of the cultures.

Neither 12.5 per cent of phenylethyl-*n*-octyl sulphide, of *p*-methoxyphenyl*iso*-propyl-*n*-octyl sulphide, of phenylethylbenzyl sulphide, nor of 3: 4-methylenedioxy-phenyl-*n*-propylbenzyl sulphide reduced the number of larvae by 90 per cent.

Extract of quassia was only tested in a concentrated solution, in which the maximum amount applied was only 6.9 per cent of the faeces; this quantity reduced the number of larvae by about 60 per cent, a reduction which could be explained by the altered physical condition of the cultures.

The addition of 5.0 per cent of " sulphoacetamide soluble " did not produce a 90 per cent reduction of larvae, neither were 1: 20 nor 1: 50 aqueous solutions effective.

Lauth's violet (chromotropic acid)$_2$ as a 1: 100 aqueous solution had little effect on the numbers of larvae.

Neither the sodium salt of penicillin, containing 100,000 units, of which over 90 per cent were penicillin G, nor an atmosphere saturated with carbon monoxide, reduced the numbers of the larvae to any appreciable extent.

Discussion

Where the difference between the amount required to kill 90 per cent and 99.9 per cent is large, it can usually be concluded that the chemical must be in intimate contact with the eggs or larvae in order to kill them. When the chemical, in contact with horse faeces, produces a lethal gas, the quantity that kills 90 per cent may also kill 99.9 per cent. However, one culture with an unusually high count of larvae can reduce the apparent lethal value of a chemical, and therefore any unexpected order of potency should, if possible, be considered in comparison with the same chemicals in a different state.

In this technique the chemicals are in the presence of a comparatively large quantity of organic matter; therefore the chemical may occasionally have been altered before the larvae hatched from the eggs. Furthermore at 25° C. a few chemicals may volatilize before the eggs have made much development.

With most compounds, whether applied solid or undiluted or in solutions, there is generally a fairly close parallelism in the order of potency for a 90 per cent and for a 99.9 per cent kill. Brief notes on the many types of chemical tested are given below.

Inorganic compounds

Data for these chemicals are given in Table I. The majority of the metallic compounds tested were more effective in dilute solutions, both for a 90 and a 99.9 per cent kill, than in more concentrated solutions or in the solid state, probably because most of these chemicals have to be in intimate contact with the eggs or larvae in order to kill them. The majority of these compounds are, to some degree, larvicides. The metallic iodides and mercury compounds generally were the most potent, but most of the sulphates had low lethal values. The chlorides and nitrates had only moderate larvicidal activity. The same amounts of sodium, potassium, cupric, and calcium nitrates, applied as solids, were required to produce a 90 per cent kill, viz. 3.7 per cent. With the exception of ferrous

and ferric chlorides, approximately twice the amount of solid metallic chlorides were required to produce a 99.9 per cent kill as were required for a 90 per cent kill.

Sodium compounds.—The most effective of the solid sodium compounds was sodium iodide; the next most effective was sodium fluoride. In solution sodium iodide was again the most effective, but sodium silicofluoride was comparable with sodium fluoride. Sodium tetraborate, both as a solid and in solutions, produced a 90 per cent kill. The low lethal value of sodium hydroxide was surprising, considering that it has been recommended as a disinfectant against hookworm larvae; however, it is one of the chemicals which sometimes makes third stage larvae exsheath and which sometimes kills them after they have developed. Sodium chloride, which has frequently been used against hookworm larvae, is only a moderately effective larvicide. Sodium bicarbonate and sodium hypochlorite both made some of the third stage larvae exsheath.

Solid sodium silicofluoride in practically all the cultures allowed some of the larvae to develop to the third stage before killing them; this characteristic was less marked when this chemical was added in solution. Sodium tetraborate applied as a solid in some cultures allowed the larvae to develop before they were killed, but only in a very few cultures did this occur when solutions of this chemical were applied. Solid sodium chlorate was four times as potent as solid potassium chlorate for a 90 per cent kill, and nearly six times for a 99.9 per cent kill.

Potassium compounds.—Of the solid potassium compounds, potassium iodide was the most lethal, and although it was about as effective as sodium iodide in producing a 90 per cent kill, it was more potent in producing a 99.9 per cent kill; in solutions these two compounds were comparable to each other. In the concentrations tested, potassium iodide and iodate were about equal and were the most lethal of the potassium compounds examined. Potassium iodate was 300 times as effective as potassium chlorate for 90 per cent and 56 times for 99.9 per cent kills; again indicating the possible effect of the iodine atom in the molecule.

Potassium chlorate appeared to be much less effective than sodium chlorate, and potassium hydroxide was slightly less effective than sodium hydroxide. Potassium hydroxide was like sodium hydroxide in that it sometimes made the larvae exsheath after the second ecdysis. Especially when applied as a solid, it sometimes killed the larvae after they had reached the third stage.

" Muriate of potash " (two grades) and potassium nitrate, both of which are used as fertilizers, gave similar results as larvicides. Kainite which was slightly more effective, and is also used as a potash fertilizer, is included in the magnesium compounds. Potassium sulphate, also a fertilizer, was rather less effective. Applied as a solid, potassium permanganate killed many of the larvae after they had reached the third stage and made some of the others exsheath, but it is a weak larvicide.

Ammonium compounds.—Included in the ammonium compounds are some nitrogenous fertilizers. All the ammonium compounds gave a 99.9 per cent kill when 10 per cent, or less, was added to the cultures. Ammonium iodide was the most effective, whether applied dry or in solution. It was found that to produce a 99.9 per cent kill ammonium sulphate, applied dry, was twice as lethal as sodium sulphate and three times more effective than potassium sulphate. When ammonium thiocyanate was applied either as a solid or in solutions in many of the cultures some of the larvae died after reaching the third stage. This happened in a few of the cultures treated with ammonium sulphamate, but fewer larvae died.

Copper compounds.—Of the four copper compounds tested copper sulphate, which is used as an anthelmintic, was the least effective. Cupric chloride was almost twice as lethal as the cuprous salt in producing 90 and 99.9 per cent reductions in the numbers of larvae. These copper compounds, however, killed many of the larvae only after they had developed

to the infective stage (see Table IV); this occurred in the majority of the cultures to which these compounds were added in sufficient quantities.

Magnesium compounds.—Magnesium borate was the most effective of the few magnesium compounds tested; there was a tendency for this chemical to kill some of the larvae after they had developed.

Calcium compounds.—Most of the calcium compounds examined had little lethal value, although many farmers consider lime to be a larvicide, even on pastures. The heat of slaking is, of course, lethal, but otherwise only large quantities kill the larvae and then usually after they have developed.

As would be expected, calcium cyanide is very lethal, and was the most effective of the calcium compounds tested; there is sufficient moisture in faeces to make it release hydrogen cyanide. One per cent of calcium arsenate produced a 90 per cent reduction in the number of larvae, but 20 per cent was required to produce a 99.9 per cent reduction; however in most of the cultures treated with more than 1 per cent, only a few larvae developed and some of them died quickly. In some of the cultures treated with solid and very concentrated solutions of calcium chloroacetate some of the larvae died after they had reached the third stage.

Zinc compounds.—In common with the other metallic iodides which were tested, zinc iodide was the most effective of the zinc compounds, whether it was applied as a solid or in solution.

All four zinc compounds tested, including zinc iodide in very concentrated solutions, killed many of the larvae after they had reached the third stage. Zinc sulphide was very much less potent in reducing the numbers or in killing them after they had developed than zinc sulphate, which was one of the most effective sulphates.

Mercury compounds.—All the mercury compounds were lethal, especially ethyl-mercuric chloride. The larvicidal effect appeared to be depressed somewhat by the introduction of an ethoxyl group as was shown by the fact that ethyl-mercuric chloride was 15 times (for a 99.9 per cent kill) and 6 times (for a 90 per cent kill) as potent as ethoxyethyl-mercuric chloride, which had the same value as the unsubstituted mercuric chloride for a 90 per cent kill.

It is surprising that mercuric iodide was the least effective of the pure mercury compounds tested. This may be due to its insolubility. Levine (1951a) found that 0.011 per cent mercuric iodide produced a 99 per cent kill. We found that 2.5 per cent gave a 99 per cent reduction in the numbers of larvae, but that 1 per cent killed all the larvae which reached the infective stage, and considerably less killed most of them. In a few of the cultures treated with mercury compounds some of the larvae died after they had reached the third stage. In a few of the cultures treated with mercuric chloride, this chemical seemed to have had a selective lethal action as only a few of the larger strongyloid larvae were alive. Ethoxyethyl-mercuric chloride also showed some selective lethal activity.

Boron compounds.—Ortho-boric acid did not differ much in its lethal potency from sodium tetraborate and magnesium borate.

Carbon monoxide.—Sealing the jars containing the cultures is in itself lethal to developing larvae; it was therefore difficult to be sure that carbon monoxide remained in contact with the eggs and larvae for any length of time; however this gas did not appear to be lethal.

Sulphur.—There seemed to be a tendency for the numbers of larvae recovered from the cultures treated with sublimed sulphur to be greater than those from the controls. This can happen with a fungicide, which is not a larvicide, if there are fungi which parasitize larvae present in the cultures. In a few cultures to which nematocidal fungi were added the number of larvae was reduced by over 90 per cent in a few weeks.

Iodine.—Some iodine compounds have already been discussed; others will be in later sections (see Tables I and II). In the three samples which were tested, iodine in colloidal form lost some of its larvicidal potency.

Manganese compounds.—Neither manganous chloride nor manganous sulphate have a high larvicidal value. Applied in solid form the chloride was two or three times as effective as the sulphate; in solutions the differences were less. Manganous sulphate was one of the more effective sulphates. In a few of the cultures treated with both compounds some of the larvae died after they had developed to the third stage.

Iron compounds.—Ferrous iodide was the most potent of the iron compounds tested, but generally their larvicidal potency was low. Added to the cultures as solids, ferrous and ferric chlorides and ferrous and ferric sulphates were very similar in their effects. Some of the larvae in many of the cultures treated with the chlorides and sulphates of ferrous and ferric iron died after they had developed to the third stage, especially in the cultures treated with the ferrous compounds.

Cobalt and nickel compounds.—Cobalt and nickel chlorides were the only salts of these metals tested; they had somewhat similar lethal values. Nickel chloride had a greater tendency to kill the larvae after they had reached the third stage, but the numbers killed were never great, although quite small percentages killed a few.

Organic compounds (aliphatic)

The larvicidal values of the organic compounds are given in Table II.

Halogen derivatives of paraffins.—In this group are several chemicals which are used for the control of plant nematodes. Only a few of these compounds are appreciably soluble in water. Those which were tested in solution showed few significant differences from their values when applied undiluted. The most noteworthy feature of these derivatives is the lethal effect of the iodides (see Table III). The corresponding bromides were less lethal, but still had a considerable effect. The corresponding chlorides tested were found to be less effective than the bromides. Ethyl chloride (b.p. 12° C.) appeared to have a very low value, but it may have dispersed before some of the eggs had hatched.

Chloropicrin (nitrochloroform) affords an example of the apparent effect of a nitro-group. The substitution of a nitro-group for the hydrogen atom in chloroform seems to increase lethal activity 60 times for a 90 per cent kill and nearly 200 times for a 99.9 per cent kill. The amounts of chloropicrin required for 90 and 99.9 per cent kills were the same.

A comparison of carbon tetrachloride with carbon tetrabromide shows the latter compound to be nearly 70 times as effective for 90 per cent and almost 240 times for 99.9 per cent kills, again showing the influence of bromine in organic compounds. The results with carbon tetrabromide are sufficiently outstanding to justify further investigation into its nematocidal properties.

A comparison of the normal alkyl bromides is interesting. The percentage of chemical required to sterilize the faeces has been plotted against the number of carbon atoms in Fig. 1; the graphs for 90 and 99.9 per cent reductions are similar. The maximum quantity of chemical required was reached with *n*-amyl bromide followed by a sharp fall to *n*-hexyl bromide.

n-Butyl bromide was 12 times as powerful as *tert.*-butyl bromide for a 90 and 5 times for a 99.9 per cent kill.

A comparison of methylene dichloride, chloroform, and carbon tetrachloride shows that there is a decrease in potency with an increase in the number of chlorine atoms. In the corresponding bromo-compounds, the potency increases with the number of bromine atoms.

The iodine compounds are more effective than the corresponding bromine compounds, which, in turn, are more so than the corresponding chloro-derivatives (cf. alkyl halides).

Ethylidene dichloride is less effective than ethylene dichloride. DD, a mixture of 1 : 3-dichloropropylene, 1 : 2-dichloropropane and higher chlorides, had approximately the same larvicidal value whether applied undiluted or in the naphtha emulsion.

Iodoform was the only compound of this group which allowed some of the larvae to develop before they died; this happened whether the iodoform was mixed in the faeces or suspended above them; in the latter case larger quantities were required.

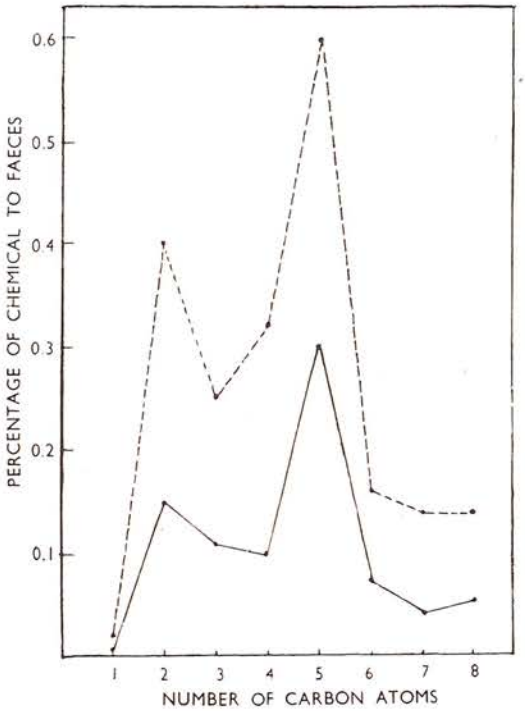

FIG. 1.—A graph to show the relationship between the number of carbon atoms in the *n*-alkyl bromides and the percentage of chemical required to produce a 90 per cent kill (continuous line) and a 99.9 per cent kill (broken line).

Halogen derivatives of ethylene.—An increase in the number of chlorine atoms results in increased potency in the chlorine derivatives of ethylene (cf. the chlorine derivatives of the paraffins). *Cis*- and *trans*-dichloroethylene were about equally effective in producing a 90 per cent kill, but the *cis*-isomer appeared to be slightly more powerful than the *trans*- for a 99.9 per cent kill. It may be that the proximity of the chlorine atoms to one another in the *cis*-compound is a contributory factor. The values for perchloroethylene were the same for 90 and 99.9 per cent kills.

Alcohols (*methyl alcohol series*).—It was found that effectiveness in producing a 90 per cent kill increased as the series of normal alcohols was ascended. *n*- and *iso*-propyl alcohols and *tert.*-butyl alcohol had the same value for a 90 per cent kill (1.5 per cent).

For a 99.9 per cent kill, methyl and ethyl alcohols were of equal effectiveness (5.0 per cent), and *n*-propyl, *n*- and *tert.*-butyl alcohols also had the same larvicidal values (1.5 per cent). The most interesting feature, however, was the greater larvicidal effect of *n*-propyl, *n*-butyl, and *tert.*-butyl alcohols, which were each twice as lethal as their *iso*-isomers. There were no significant differences whether these chemicals were applied undiluted or in aqueous solutions.

The addition of some compounds to the cultures occasionally produces third stage larvae which, although alive, are shrunken; a few of these larvae were found in cultures treated with methyl alcohol.

Ethers.—The two substituted diethyl ethers were more powerful larvicides than the unsubstituted compound. The substitution of two chlorine atoms for two hydrogen atoms increased the effect 30 times for a 90 per cent kill, and 36 times for a 99.9 per cent kill. In some cultures treated with dichlorodiethyl ether a few of the larvae died after they reached the third stage.

Sulphides.—Tetramethylthiuram monosulphide and the corresponding disulphide, both of which were applied in slight suspension, were very much more effective when added to the cultures in this way than when they were added dry. In most suspensions the disulphide, although 54 per cent of the mixture added was an inert dispersal agent, was more effective than the monosulphide in reducing the numbers of the larvae; but in the cultures treated with the monosulphide there was a greater tendency for many of the larvae to die after they had reached the third stage.

Formaldehyde derivative.—A few of the larvae in cultures to which hexamethylene tetramine had been added died after they had reached the third stage.

Esters.—Ethyl iodoacetate, like other lachrymators, has a fairly high larvicidal value. With the exception of this compound, the other esters which were tested all allowed some larvae to reach the third stage before killing them; this characteristic was very marked when hexaethyl tetraphosphate, diethyl-*p*-nitrophenylthiophosphate or Teepol X were added undiluted.

Amines.—In many of the cultures to which dimethylamine had been added as a 25 per cent aqueous solution dead eggs were found, and a few were found in four cultures to which weaker solutions had been added. When this compound was added to the cultures in small quantities some of the larvae reached the third stage before they were killed.

Cetyltrimethylammonium bromide, a wetting agent, and the 20 per cent solution of alkylammonium bromides, dissolved in methylated spirit, both killed some larvae slowly, so that they had reached the third stage before they died, but this occurred much more frequently with the former.

Allyl compounds.—The allyl compounds tested were very effective larvicides, particularly allyl isothiocyanate (a lachrymatory liquid) and allyl iodide, which were approximately equal. Allyl isothiocyanate and allyl alcohol, the two allyl compounds which were tested as aqueous solutions, showed very little difference in value whether applied undiluted or in solutions. When the allyl halides were compared, it was found, as with the alkyl halides, that allyl iodide was more powerful than the bromide, which in turn was more effective than the chloride. Compared with alcohols of the methyl alcohol series, allyl alcohol (a lachrymatory liquid) was much more potent.

Amino acid.—The addition of cystine to the cultures even in large proportions only reduced the number of larvae slightly, but a few more died after they had reached the third stage.

Carbonic acid derivatives.—Both zinc and ferric dimethyldithiocarbamates were much more effective applied in suspension than added dry. Small quantities of both compounds markedly reduced the numbers of larvae which reached the third stage and quite small quantities killed some of the larvae after they had developed; however, not even large quantities of the dry compounds prevented all the larvae developing to the third stage or killed all that did so.

The substitution of a sulphur atom for oxygen in urea increased the effect eight times for 90 per cent and twice for 99.9 per cent reductions in the number of larvae, when the

compounds were applied as solids. In solution, thiourea was generally more effective, and, in addition, many of the larvae in cultures treated with thiourea solutions died after they had developed to the third stage.

The frequency with which some larvae developed to the third stage and then died was marked in cultures treated with methylguanidine sulphate, diphenyl guanidine, and creatinine in the more concentrated solutions; this also occurred in the more concentrated solutions of guanidine hydrochloride, *as*-dimethylguanidine sulphate and creatine, but to a less marked extent.

Ureides and purine derivatives.—Although the addition of 50 per cent of allantoin in the dry state to the cultures did not reduce the number of third stage larvae found by more than about 99.7 per cent, the addition of only 1.25 per cent ensured that they had all died after they had developed to the third stage.

Both xanthine and guanine added to the cultures of fresh horse faeces as solids killed some of the third stage larvae.

Organic compounds (carbocyclic)

Hydrocarbons.—The xylenes (*ortho*-, *meta*, and *para*-mixed) and *p*-cymene were equally potent and very effective larvicides, more so than toluene, which was more effective than benzene. It would appear that an increase in the number of alkyl groups increases the larvicidal effect.

Halogen derivatives.—Chloro-, bromo-, and iodo-benzene, *o*- and *p*-dichloro-benzene, and cyclohexyl bromide were quite effective. Benzene hexachloride (containing a mixture of isomers) and its α-, β-, γ-, and δ-isomers separately, and DDT were all tested as solids, but had low or no larvicidal values. However, in many of the cultures to which 3.75 per cent or more of benzene hexachloride was added some of the larvae died after reaching the third stage, but even 50 per cent of benzene hexachloride only reduced the number of larvae which were extracted from the cultures by about 80 per cent.

Chloro-, bromo-, and iodo-benzenes were tested in very dilute solutions, but they did not affect the eggs or larvae.

Although the addition of 50 per cent of 2:3:5:6-tetrachloronitrobenzene to fresh horse faeces did not quite reduce the number of larvae recovered by 99.9 per cent, yet the addition of only 1.9 per cent ensured that some of the larvae died after reaching the third stage.

Mercury compounds.—The two mercuric compounds confirm the lethal effect of the mercury atom, which was noticed in the inorganic and aliphatic compounds of mercury. Both tended to kill some of the larvae after they had developed.

Amino-compounds.—Aniline is an effective larvicide, but the other compounds, which were tested, were not so effective in reducing the numbers of the larvae. However, diphenylamine in small quantities affected the viability of the larvae; for example, in some cultures treated with 0.5 per cent, and over, a considerable number of larvae were alive when they were extracted from the cultures, but ten days later all or practically all, had died. Diphenylamine tended to darken the larvae.

Azo-compounds.—In cultures treated with azobenzene and with aminoazobenzene hydrochloride some larvae died after they had reached the third stage. Aminoazobenzene hydrochloride makes the larvae sluggish; therefore it is probable that a lower proportion of live larvae than normal was recovered from the cultures. This compound occasionally stains live larvae.

Phenols.—The relative effectiveness of the phenols was increased by dilution with water. The low boiling-point phenols were more effective than the less volatile phenols.

Filix mas emulsion and 4-*n*-hexylresorcinol, two compounds with specific anthelmintic properties, have low larvicidal potencies. Apart from chemical reasons this apparent anomaly may be due to the physical condition of the Filix mas emulsion and to the poor solubility of 4-*n*-hexylresorcinol (1 : 2,000) in water, which would only allow it to penetrate the faeces slowly. There is, perhaps, confirmation of this in the fact that many larvae in cultures treated with 4-*n*-hexylresorcinol develop to the third stage before being killed. Resorcinol, on the other hand, is quite effective; so are phenol, sodium 2 : 4 : 5-trichlorophenate, *o*-nitro-phenol, and thymol.

The same amount of *o*-nitro-phenol is required for a 90 as for a 99.9 per cent kill. This compound is 5 times as effective for a 90 per cent and 20 times as effective for a 99.9 per cent kill as the *para*-isomer. Sodium 2 : 4 : 5-trichlorophenate has about the same effect as phenol, but the larvicidal effect is considerably reduced in sodium *o*-phenylphenate and still more so in sodium *m*-chloro-*p*-phenyl phenate. The introduction of a phenyl group may be the contributory factor.

Among the phenols were compounds which appeared to kill the eggs and many which allowed some of the larvae in the cultures to develop before killing them. These apparent contradictions may be due to the very local action of some of these compounds. For example, when the chemicals were mixed in the cultures they would immediately be in contact with some eggs; during development, more larvae would come in contact with the chemicals, but some might not do so until they had developed to the third stage.

Ketone.—*p*-Chloroacetophenone is another lachrymatory compound, but its comparatively low vapour pressure may account for the fact that its larvicidal potency is lower than that of the other lachrymators, and for the fact that some larvae in the cultures were able to develop to the third stage before being killed.

Monobasic acid.—Bloomfield (1949) found that benzoic acid was the only constituent of urine which killed eggs, preinfective and infective larvae. In the present investigation, the addition of 50 per cent of benzoic acid only reduced the number of larvae by 97 per cent; 30 per cent was the minimum percentage which killed all the larvae which reached the third stage in all the cultures although in some cultures all the larvae were killed by smaller quantities. This suggests that this compound must be in close contact to be lethal to eggs or larvae.

Esters of dibasic acids.—Dimethylphthalate had a very low lethal value, but it was more potent than the dibutyl ester, and killed many of the larvae which developed to the third stage, which dibutylphthalate did not do.

Triphenylmethane dyes.—Added as solids these dyes have a low potency as larvicides, although some of them are recognized anthelmintics against some nematodes which parasitize man. Gentian violet was much more effective in solution than in the solid state in producing a 90 per cent kill, but did not produce a 99.9 per cent kill. In some of the cultures treated with these dyes some of the third stage larvae, both live and dead, were stained; the staining was particularly frequent in the larvae from cultures treated with magenta, methyl violet, and gentian violet. As some of the larvae died fairly soon after reaching the third stage (see Table IV) it seems probable that these dyes have a slow lethal action on the free-living stages in faeces at 25° C.

Terpenes.—Turpentine, essential oil of *Artemisia maritima*, containing 65 per cent β-thujone and 16 per cent 1 : 8-cineole, and oil of chenopodium were effective larvicides, but santonin was of no value. This may be caused by its physical condition, although as a 1 : 500 aqueous solution 25 c.c. in 40 g. of fresh faeces only caused a 70 per cent reduction in the number of third stage larvae which were found; it is really a vermifuge.

1 : 8-Cineole and β-thujone were tested separately and found to be effective. β-Thujone had the same value for 90 and 99.9 per cent kills, and was 6 times more potent than 1 : 8-cineole for a 99.9 per cent kill.

Naphthalene and β-naphthol.—Judged by the numbers of larvae which developed to the third stage, the larvicidal effect of naphthalene was weakened very considerably by the introduction of the hydroxyl group in the β-position. This was rather unexpected as the introduction of a hydroxyl group sometimes increases anthelmintic activity. Many of the third stage larvae died in the cultures treated with β-naphthol, but even for this delayed killing effect considerably more β-naphthol was required than was necessary to produce a 99.9 per cent reduction in the numbers of larvae in the naphthalene treated cultures.

Organic compounds (heterocyclic)

Benzthiazole.—In practically all of the cultures treated with 2.5 per cent, or more, of 2-mercapto-benzthiazole some of the larvae which reached the third stage rapidly died, although 35 per cent was required to produce even a 90 per cent reduction in the number of larvae recovered from the cultures.

Pyridine derivatives.—Pyridine and derivatives were found to be effective larvicides and had values ranging from 0.09 to 0.23 per cent for 90 per cent and from 0.17 to 0.25 per cent for 99.9 per cent kills. The lethal values of these chemicals were little changed on dilution with water.

Quinoline and derivative.—In many of the cultures treated with undiluted quinoline and with concentrated solutions of 8-hydroxyquinoline sulphate many larvae died after they had reached the third stage; some larvae died in a few of the cultures treated with these two compounds in less concentrated solutions.

Acriflavine.—Acriflavine sometimes killed the larvae after they had reached the third stage, yet at other times the third stage larvae were deeply stained but were apparently unharmed.

Benzthiazine derivatives.—These derivatives had very little larvicidal value; the simplest compound, 3-keto-2 : 3-dihydrobenz-1 : 4-thiazine, was the most lethal. In the cultures treated with the aminohydrochloride many of the larvae died after they had reached the third stage; this compound adhered to their sheaths.

Phenoxthine.—Although the addition of very small quantities of phenoxthine to cultures reduced the numbers of larvae which reached the third stage very considerably, and many of the larvae which did reach that stage died, yet even 50 per cent of phenoxthine added to the cultures did not prevent some larvae developing. In twenty out of forty-five cultures treated with 2.5 per cent, or more, of this compound all the larvae were dead.

Phenothiazine and derivatives.—Mercurated phenothiazine was a powerful larvicide, but phenothiazine and the other phenothiazine derivatives applied as solids were not effective larvicides. Phenothiazine, phenothiazone, and methylene blue were more effective in producing a 90 per cent kill in solution or suspension than when applied dry, but none of these chemicals produced a 99.9 per cent kill. The fact that in each culture of horse faeces there are larvae of many species probably decreases the chance of a 99.9 per cent kill with any chemical which is a specific larvicide; as an anthelmintic phenothiazine is selective.

Lauth's violet was not particularly effective, but when coupled with phenol, its potency was slightly increased. Other couplings with Lauth's violet decreased its value; but many of the larvae died after they reached the third stage in cultures treated with Lauth's violet⇄(β-naphthol)$_2$, some in the cultures treated with Lauth's violet⇄(2-hydroxy-3-naphthoic acid)$_2$, with phenothiazone and with over 30 per cent of methylene blue.

Alkaloids.—Of the alkaloids added as solids to the cultures arecoline hydrobromide was the most effective. Nicotine sulphate (40 per cent solution) diluted twenty to fifty times with water was considerably more effective than in the more concentrated solution.

Plant products.—The plant products which were tested had very little larvicidal value. The crushed leaves were tried in order to determine whether or not they might slowly release sufficient hydrogen cyanide to be of value in destroying plant nematodes, if crops with high nitrogen contents were ploughed in as green manure.

Urines and manure.—Although fresh poultry manure and urines have a low larvicidal value, it has been found that they have a practical value for killing larvae in manure heaps and in night-soil.

Physical properties

In general there appeared to be no correlation between boiling-points and larvicidal effect. In the inorganic compounds there seemed to be a general tendency for larvicidal effect to increase with solubility in water, but there were exceptions. In the organic compounds no increase in larvicidal effect was observed with increase of solubility, when the chemicals are taken as a whole. It would appear that, unless the chemical has to be in intimate contact with the eggs or larvae, solubility in water is of secondary importance. No simple correlation exists between larvicidal action and molecular weight, vapour pressure or parachor.

Conclusions

Many chemicals were applied to fresh horse faeces in a confined space at a temperature of about 25° C.; from the results it has been possible to draw some conclusions on their effect as larvicides against the free-living stages of Sclerostomes.

1. Of the metallic compounds which were tested, those of mercury were the most potent larvicides.
2. The metallic iodides are generally the most potent of the inorganic salts; compounds of fluorine and boron are less effective larvicides.
3. The nitrates have comparable larvicidal values to the chlorides, both of which are more potent than the sulphates.
4. The ammonium compounds are larvicidal and have a practical value in killing larvae in night-soil and middens.
5. The copper compounds are notable for the slowness with which they kill Sclerostome larvae.
6. Calcium compounds, in spite of their reputation, are very weak larvicides.
7. Sulphur has no larvicidal action.
8. Most inorganic compounds are more effective when applied in solution than as solids.
9. Iodo-derivatives of the paraffins are very lethal, the order of potency of halogenated paraffins being iodo>bromo>chloro.
10. The substitution of a nitro-group for the hydrogen atom in chloroform considerably increases its larvicidal value.
11. The larvicidal value of carbon tetrabromide is high; this chemical should be tested against other helminths.
12. *n*-Amyl bromide is the least effective of the normal alkyl bromides in the series methyl to octyl.

13. *n*-Butyl bromide is more lethal than *tert.*-butyl bromide.

14. In the halogen derivatives of the paraffins there is a decrease in larvicidal potency with an increase in the number of chlorine atoms, but an increase in potency with an increase in the number of bromine atoms.

15. In the halogen derivatives of ethylene an increase in the number of chlorine atoms increases the larvicidal potency.

16. The effectiveness of the normal alcohols to produce a 90 per cent reduction in the number of larvae increases as the series of alcohols is ascended.

17. *n*-Propyl, *n*-butyl, and *tert.*-butyl alcohols are more lethal than their *iso*-isomers.

18. The substitution of two chlorine atoms for two hydrogen atoms in ether considerably increased the larvicidal value.

19. Tetramethylthiuram disulphide is more effective than the monosulphide in reducing the number of larvae, but both have to be in intimate contact with the larvae to kill them.

20. Dimethylamine and diphenylamine are both ovicidal as well as larvicidal, but the latter has a peculiar delayed larvicidal action.

21. Allyl compounds are very potent larvicides, especially the isothiocyanate and the iodide. With the halides, the order of potency is I>Br>Cl.

22. Both zinc and ferric dimethyldithiocarbamates have to be in intimate contact with larvae to kill them.

23. The substitution of a sulphur atom for oxygen in urea increases its larvicidal value considerably.

24. Many of the carbonic acid derivatives have a slow lethal action.

25. In the aromatic hydrocarbons an increase in the alkyl groups increases the larvicidal values.

26. Chloro, bromo- and iodobenzene, *o*- and *p*-dichlorobenzene are quite potent larvicides, but benzene hexachloride has a very low larvicidal value, and 2: 3: 5: 6-tetrachloronitrobenzene is a very slow killer.

27. Azobenzene and aminoazobenzene hydrochloride also seem to have a delayed lethal effect.

28. A number of phenolic compounds are effective larvicides, but several of them, including sodium 2: 4: 5-trichlorophenate, sodium-*o*-phenyl phenate, and sodium *m*-chloro-*p*-phenyl phenate, allow many of the larvae to develop to the third stage before they are killed. The introduction of a phenyl group reduces the larvicidal value of sodium 2: 4: 5-trichlorophenate. *o*-Nitro-phenol is much more effective than the *para*-isomer.

29. Some of the triphenylmethane dyestuffs also have a delayed lethal action.

30. The value of β-thujone should be tested against other helminths.

31. β-Naphthol is a less effective larvicide than naphthalene.

32. Pyridine and its derivatives are effective whether applied undiluted or as solutions.

33. As larvicides the benzthiazine derivatives have low values.

34. Phenoxthine has a slow lethal effect.

35. Phenothiazine and its derivatives, except mercurated phenothiazine, have low larvicidal values, but were more effective in solutions.

36. Urines and poultry manure, because of their availability on farms and their value as fertilizers, can be used in well-made middens to kill bursate nematode larvae, although their larvicidal potency is low.

37. The lachrymators, which were tested, have high larvicidal values.

38. Solubility is no guide to larvicidal effect; except in a number of inorganic compounds, where larvicidal effect increases with increase of solubility in water.

39. There is no correspondence between boiling-points, molecular weights, vapour pressures and parachors, and larvicidal values.

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BY

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Azo-dyestuffs have been prepared from 6-amino-2,3-dihydro-3-ketobenzo-1,4-thiazine by coupling with various hydroxy-compounds and with anthranilic acid. Some of them have shown depressant effects on *Ascaris lumbricoides* and liver fluke (*Fasciola hepatica*) *in vitro*, and gave good shades on wool. All the colours were fast to light.

Mackie and *Raeburn* [1]) prepared a number of 2,3-dihydro-3-ketobenzo-1,4-thiazine derivatives and found [2]) that almost all the compounds had a paralysant effect on liver fluke (*Fasciola hepatica*) when tested *in vitro*. Since the chemotherapeutic value of certain azo-dyestuffs has been noted on several occasions, it was considered possible that azo-dyestuffs obtained from diazotised 6-amino-2,3-dihydro-3-ketobenzo-1,4-thiazine and various hydroxy-compounds and anthranilic acid as coupling components might show anthelmintic effects. Consequently a series of these azo-dyestuffs were prepared and tested against liver fluke and the large round worm (nematode), *Ascaris lumbricoides, in vitro*.

As a few of the dyestuffs had bright colours, dyeings were made with wool and their fastness to light examined.

Experimental Part.

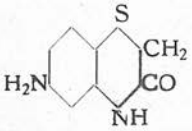
Some microanalyses by Drs. *Weiler and Straus*, Oxford, England.
Decomposition temperatures and m.p.s are uncorrected.

Preparation of azo-dyestuffs from diazotised 6-amino-2,3-dihydro-3-ketobenzo-1,4-thiazine and various coupling components.

6-Amino-2,3-dihydro-3-ketobenzo-1,4-thiazine I), *Mackie* and *Raeburn* [1]), (4.5 g) was diazotised below 10° and added to the calculated quantity of the hydroxy-

[1]) *A. Mackie* and *J. Raeburn*, J. Chem. Soc. **1952**, 787.
[2]) *A. Mackie* and *J. Raeburn*, Brit. J. Pharmacol. **7**, 219 (1952).

component dissolved in excess aqueous sodium hydroxide below 10°. The coupling with anthranilic acid was carried out below 10° in neutral solution.



(I)

When coupling was complete (6—12 hr), the precipitate was filtered off, washed with absolute ethanol followed by acetone, cf. *Sah* [3]), finally dried and ground.

The coupling components were phenol (A), β-naphthol (B), salicylic acid (C), phloroglucinol (D), 4-n-hexylresorcinol (E), thymol (F), 2-hydroxy-3-naphthoic acid (G), F-acid (2-naphthol-7-sulphonic acid) (H), 8-hydroxy-quinoline (I), 1-phenyl-3-methyl-5-pyrazolone (J), and anthranilic acid (K). See table I. The coupling with thymol required further purification by dissolving in ethanol-acetone, refluxing the solution with animal charcoal, and reprecipitating with water.

As far as is known, with the exception of the coupling with β-naphthol [4]), these dyestuffs have not been prepared previously. *Friedlaender* and *Chwala* appear to have carried out a test-tube experiment only and do not describe the dyestuff in detail.

Biological testing.

The dyestuffs were tested *in vitro* against the anterior preparations of *Ascaris lumbricoides*, employing *Baldwin*'s kymographic technique [5]), and liver fluke, cf. *Chance* and *Mansour* [6]); *Mackie* and *Raeburn* [2]). The maximum concentration employed was 1 : 1.000, either as emulsion or suspension, and the temperature was maintained at 37—38° C.

Results and Discussion.

None of the dyestuffs examined had a paralysant effect either on liver fluke or on *Ascaris*, but a number of them had a depressant action. It would appear, therefore, that the lengthening of the side chain in this way decreases any anthelmintic effect considerably, since most of the derivatives of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine examined by *Mackie* and *Raeburn* [2]) against liver fluke were paralysants. Some of these dyestuffs were sparingly soluble in water and were used in suspension; this may have influenced the activity. The results are summarized in table I.

It is interesting to note that couplings with β-napthol (B) and 4-n-hexylresorcinol (E) show only depressant effects on both parasites, and with thymol (F) a depressant effect on liver fluke and no effect on *Ascaris*, since *Baldwin* [5]) showed that these components

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[3]) *P. P. T. Sah*, Rec. trav. chim. **69**, 1414 (1950).
[4]) *P. Friedlaender* and *A. Chwala*, Monatsh. **28**, 277 (1907).
[5]) *E. Baldwin*, Parasitology **35**, 89 (1943).
[6]) *M. R. A. Chance* and *T. E. Mansour*, *Brit.* J. Pharmacol. **4**, 7 (1949).

Table I.

Properties and analytical data of azo-dyestuffs from 6-amino-2,3-dihydro-3-ketobenzo-1,4-thiazine.

Component	Decomposes at	Yield %	Formula	% Nitrogen		Colour on Wool	Effect on	
				Found	Calculated		Ascaris	Liver Fluke
A	262—3°	79	$C_{14}H_{10}O_2N_3SNa$	13.7	13.7	Yellow	None	Depressant
B	241—2°	88	$C_{18}H_{12}O_2N_3SNa$	11.5	11.8	Orange	Depressant	,,
C	280°	97	$C_{15}H_9O_4N_3SNa_2$	10.8	11.3	Yellow	,,	,,
D	> 300°	94	$C_{14}H_8O_4N_3SNa_3$	10.9	11.0	Brown	None	,,
E	226—8°	55	$C_{20}H_{21}O_3N_3SNa_2$	10.1	9.8	Orange	Depressant	,,
F	206—8°	40	$C_{18}H_{18}O_2N_3SNa$	11.7	11.6	Light Orange	None	,,
G	276—7°	90	$C_{19}H_{11}O_4N_3SNa_2$	9.5	9.9	Light Brown	,,	None
H	> 300°	52	$C_{18}H_{11}O_5N_3S_2Na_2$	9.4	9.2	Crimson	Depressant	Depressant
I	252—3°	94	$C_{17}H_{11}O_2N_4SNa$	15.3	15.6	Yellowish Brown	,,	,,
J	272—4° (melts)	88	$C_{18}H_{15}O_2N_5S$	19.3	19.2	Light Yellow	None	,,
K	175—6° (melts)	60	$C_{15}H_{13}O_3N_4ClS$	15.6	15.4	Yellowish Brown	,,	,,

per se produced paralysant effects on *Ascaris*, and *Chance* and *Mansour* [6]) found that they were lethal towards liver fluke. Both β-naphthol and thymol have been, and 4-n-hexylresorcinol still is, used as anthelmintics.

All the dyestuffs showed very good fastness to light, and the majority gave good shades on wool.

Acknowledgements.

The authors gratefully acknowledge a grant from the Agricultural Research Council and record their thanks to Professor *Bell* for his interest in this work, to Dr. *Raeburn* for his assistance with some of the experimental work, to Dr. *Minnis* for some of the microanalyses, and to Mr. *Soulsby*, late of the Edinburgh City Abattoir for collection of the parasites.

(Received September 1st 1952).

1 Janvier 1953

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THE EFFECT OF SOME OXIDATION PRODUCTS OF PHENOTHIAZINE ON *ASCARIS LUMBRICOIDES IN VITRO*

BY

ALEXANDER MACKIE

(Arch. int. Pharmacodyn.)

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FROM THE DEPARTMENT OF CHEMISTRY, HERIOT-WATT COLLEGE, EDINBUGH

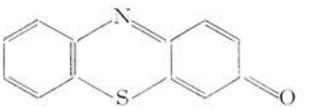
THE EFFECT OF SOME OXIDATION PRODUCTS OF PHENOTHIAZINE ON *ASCARIS LUMBRICOIDES IN VITRO*

BY

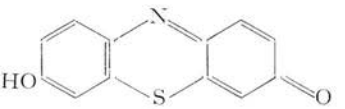
ALEXANDER MACKIE

(Received for publication 23-7-1952*)*.

The effects of the oxidation products of phenothiazine, viz., phenothiazone (I), thionol (II), and phenothiazine sulphoxide (III) on liver fluke *(Fasciola hepatica) in vitro* have been investigated by MACKIE and RAEBURN (1952), who found that (I) was lethal at a concentration of 1 : 8.000 and paralysant at 1 : 16.000, whilst (II) and (III) were only paralysant at 1 : 1.000 and 1 : 4.000 respectively.



(I)



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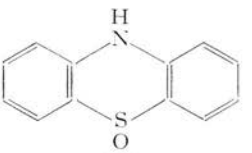

(III)

Since the effects of these compounds on liver fluke were so definite, it was considered desirable to carry out investigations *in vitro* against *Ascaris lumbricoides*, although phenothiazone administered to sheep was found to have no effect on nematodes removable by phenothiazine (COLLIER, ALLEN and SWALES, 1943).

METHODS

Preparative. — Phenothiazone, thionol and phenothiazine sulphoxide were prepared by the methods of OLIVIER and COMBE (1950), HOUSTON,

KESTER and DEEDS (1949), and BARNETT and SMILES (1909) respectively.

Biological testing. — The oxidation products were tested against *Ascaris lumbricoides in vitro* using BALDWIN's kymographic technique (1943), the maximum concentration used was 1 : 1.000, the temperature maintained at 37-8°, and the helminth preparation subjected to the action of the compound for 30-40 min. Anterior preparations of *Ascaris* were employed, since they contain the so-called "nerve ring" (cf. BALDWIN, 1943).

RESULTS AND DISCUSSION

As was found for liver fluke, phenothiazone was the most potent of the three compounds tested and produced paralysant effects on the *Ascaris* preparation at concentrations from 1 : 1.000 to 1 : 10.000, the minimum effective concentration, and showed depressant effects from 1 : 12.000 to 1 : 16.000. Thionol had no effect even at 1 : 1.000, but phenothiazine sulphoxide caused a powerful depressant effect at 1 : 1.000.

As neither amphetamine sulphate nor strychnine hydrochloride have any stimulant effect on *Ascaris*, it was difficult to ascertain whether phenothiazone was lethal or paralysant at concentrations higher than 1 : 10.000. In Table I, in which the results are summarized, it is described as paralysant at 1 : 10.000, since movement was restored after the preparation had been placed in fresh Ringer's solution, but no recovery was observed, even after 2-3 hr. in fresh Ringer, when the preparation had been subjected to concentrations of 1 : 8.000 and higher.

The kymographic record for phenothiazone is shown in Fig. 1, where the normal movement of the preparation in Ringer's solution is shown from the beginning of the experiment to the long stroke on the signal

TABLE I

Effect of oxidation products of phenothiazine on Ascaris lumbricoides in vitro

Compound	Nature of preparation	Concentration	Effect
Phenothiazone	Emulsion	1 : 10.000	Paralysant
	»	1 : 16.000	Depressant
Thionol	»	1 : 1.000	None
Phenothiazine sulphoxide	Suspension	1 : 1.000	Strong depressant

line, marked in minutes. The phenothiazone emulsion replaced the Ringer solution at the time indicated by the long stroke.

The order of potency of the three compounds is the same as that obtained for liver fluke, and if the sulphoxide had been more soluble, in all probability, a paralysant effect would have been observed at

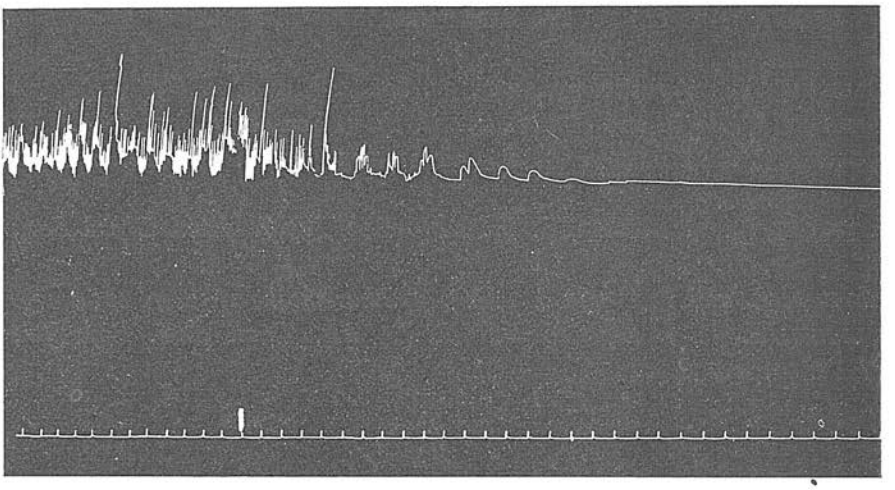

FIG. 1
Paralysant effect of phenothiazone (1 : 10.000)

1 : 1.000. It is significant that the powerful paralysant effect of phenothiazone is completely destroyed by the introduction into the molecule of a hydroxyl group in position 7 or reduction to phenothiazine, which, BALDWIN (1943) found to have no effect on *Ascaris in vitro*.

The observations recorded in this paper again indicate the desirability of carrying out further experiments *in vivo* with the oxidation products of phenothiazine, especially phenothiazone.

CONCLUSIONS

1. The oxidation products of phenothiazine, viz., phenothiazone, thionol, and phenothiazine sulphoxide, have been tested at various concentrations against *Ascaris lumbricoides in vitro*.

2. Phenothiazone had a paralysant effect at a concentration of 1 :10.000 and at 1 : 16.000 a depressant effect was observed. Thionol had no effect at 1 : 1.000 but phenothiazine sulphoxide exerted a powerful depressant effect at 1 : 1.000.

I am indebted to the Agricultural Research Council for financial assistance and to Mr E. J. L. SOULSBY, late of the Edinburgh City Abattoir, for collection of the parasites. It is a pleasure to record my thanks to Professor E. BALDWIN and Dr I. W. PARNELL for their interest in this investigation.

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A SIMPLE METHOD FOR THE DETECTION OF PHENOTHIAZINE

By

ALEXANDER MACKIE

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A SIMPLE METHOD FOR THE DETECTION OF PHENOTHIAZINE.

by ALEXANDER MACKIE*

Phenothiazine poisoning in sheep occurs so seldom that no simple method appears to be available for the detection of phenothiazine in the alimentary tract. Consequently when such a case did occur (Dunn, (3)), a method for its detection had to be evolved. It is simple and since veterinary surgeons carrying out post mortems on sheep may occasionally require to know how far down the alimentary tract phenothiazine has been carried, a description of the method is given in this paper.

Several methods for the quantitative determination of phenothiazine are to be found in the literature. Eddy and DeEds (4) and Cupples (2) described colorimetric methods for estimating phenothiazine by taking advantage of the highly coloured red compound, formed by treating ethyl alcoholic solutions with bromine water. Overholser and Yoe (6) employed palladous chloride which gave a dark blue colouration or precipitate of the complex Pd $(C_{12}H_9NS)^2Cl_2$ with acetone or ethyl alcoholic solutions of phenothiazine. Canbâck (1) determined colour density of ethyl alcoholic solutions of phenothiazine treated with potassium bromate-potassium bromide reagent and Kniaseff (5) employed cuprous chloride as a colorimetric reagent.

These methods were specifically designed for quantitative estimation and it was considered desirable to seek some other reagent which could be more conveniently used for the qualitative detection of phenothiazine in intestinal contents. Such a reagent has been found in perhydrol (30% hydrogen peroxide).

Acetone solutions of phenothiazine (10 c.c.) of different concentrations were mixed with varying amounts of perhydrol in stoppered conical flasks of 100 c.c. capacity, when a red colour, due probably to the oxidation product phenothiazone, $C_{12}H_7ONS$ developed on standing. The best results were obtained when 5 c.c. perhydrol were added to 10 c.c. of phenothiazine solution. The rate of development of the colour varied to some extent with the concentration. By this method phenothiazine could be detected in solutions containing 0.0025%. The following table illustrates typical results. The development of colour could be accelerated by boiling the mixture under reflux for 2-3 minutes, but only pale pink shades were observed with concentrations greater than 0.01%.

This method was applied to ascertain if any phenothiazine could be detected in the abomasum, the small and large intestine of the sheep examined. Samples of the contents of these organs were evaporated to dryness on the water-bath, extracted with acetone and filtered. The filtrates were refluxed with animal charcoal, when colourless solutions were obtained. On shaking these solutions with perhydrol in stoppered conical flasks and allowing to

* Department of Chemistry, Heriot-Watt College, Edinburgh, Scotland.

stand, a distinct red colour appeared with the extract from the large intestine showing the presence of phenothiazine. Only faint colouration appeared in extracts from the abomasum and small intestine.

TABLE 1

Concentration (%)	Time required for Colour Development (hr.)
1, 0.75, 0.5, 0.25	0.5*
0.1, 0.075, 0.05, 0.025, 0.01	0.5
0.0075, 0.005	1
0.0025	3 (faint coloration)
0.001	No colour after several days.

* In these cases a purple colour developed, possibly due to the formation of thionol, $C_{12}H_7O_2NS$, another oxidation product of phenothiazine.

Since phenothiazine is used as an insecticide, it is possible that this colour test may be useful for the detection of phenothiazine in spray residues.

SUMMARY AND CONCLUSIONS

Small quantities of phenothiazine may be detected by the development of the red colouration produced on addition of perhydrol (30% hydrogen peroxide.)

Phenothiazine was detected in the large intestine of a sheep by application of this test.

The author wishes to thank Dr. I.W. Parnell for his helpful criticism.

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PREPARATION OF 2:3-DIHYDRO-3-OXOBENZO-1:4-THIAZINE DERIVATIVES AS POSSIBLE ANTHELMINTICS PART II

By

ALEXANDER MACKIE

and

A. ANTHONY CUTLER

Preprinted from the Journal of the Chemical Society

November, 1953, (754), pages 3716 - 3717

PAPER No. 8

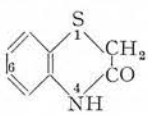
Preprinted from the Journal of the Chemical Society,
November, 1953, (754), pages 3716—3717.

754. *Preparation of 2 : 3-Dihydro-3-oxobenzo-1 : 4-thiazine Derivatives as Possible Anthelmintics. Part II.**

By Alexander Mackie and A. Anthony Cutler.

Since many derivatives of 2 : 3-dihydro-3-oxobenzo-1 : 4-thiazine (I) show distinct anthelmintic effects when tested *in vitro* against liver fluke (*Fasciola hepatica*) (Mackie and Raeburn, *Brit. J. Pharmacol.*, 1952, **7**, 219), the earlier work in this series (*idem*, *J.*, 1952, 787; Mackie and Cutler, *Rec. Trav. chim.*, 1952, **71**, 1198) has been extended, particularly to 6-substituted compounds.

The 6-bromo-, 6-methyl, and 6-*tert.*-butyl derivatives were obtained by reduction of the corresponding *para*-substituted (*o*-nitrophenylthio)acetic acids. These acids were prepared by treating the appropriate diazotised *o*-nitro-amine with mercaptoacetic acid in presence of aqueous sodium acetate and decomposing the (phenyldiazothio)acetic acids thus obtained by hot aqueous sodium carbonate (cf. Friedlaender and Chwala, *Monatsh.*, 1907, **28**, 279). Previous attempts to prepare the 6-bromo-compound either by Sandmeyer reaction or by decomposition of the diazonium perbromide resulted only in highly impure material.


(I)

6-Toluene-*p*-sulphonamido-, 6-acetamidobenzenesulphonamido-, and 6 : 7-diethoxy-derivatives of (I) were also prepared.

Analogy with known anthelmintics led to unsuccessful attempts to condense the 6-chloro-derivative of (I) with 2-diethylaminoethylamine, and the 6-amino-derivative with 10-chloroacetylphenothiazine, and to prepare the 6 : 7-dichloro-derivative of (I) from the 6 : 7-dihydroxy-compound.

The derivatives prepared showed only depressant or no effect when tested against anterior preparations of the roundworm *Ascaris lumbricoides*, but some had a paralysant effect on liver fluke (*Fasciola hepatica*) *in vitro*, the 6-bromo-derivative being very effective (1 : 20,000). Details of these tests will be published elsewhere.

Experimental.—6-*Chloroacetoamido*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*. Chloroacetyl chloride (6 g.) was slowly added to a boiling glacial acetic acid solution (150 c.c.) of 6-amino-2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine (7 g.), and the mixture refluxed for 30 min. Pouring the cooled product into cold water gave the pale brown *chloroacetoamido*-derivative, which crystallised from aqueous ethanol as colourless needles (4 g.), m. p. 240—241° (decomp.) (Found N, 11·1. $C_{10}H_9O_2N_2ClS$ requires N, 10·9%).

6-*Benzamido*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*. Benzoyl chloride (15 c.c.) was added portionwise to vigorously agitated 6-amino-2 : 3-dihydro-3-oxobenzo-1 : 4-thiazine (5 g.) suspended in aqueous sodium hydroxide (175 c.c.). The precipitated *benzamido*-derivative crystallised from acetic acid in colourless needles (3 g.), m. p. 269—270° (decomp.) (Found : N, 9·6. $C_{15}H_{12}O_2N_2S$ requires N, 9·9%).

2 : 3-*Dihydro*-3-*oxo*-6-*toluene*-p-*sulphonamidobenzo*-1 : 4-*thiazine*. A pyridine solution (30 c.c.) of the 6-amino-compound (4·5 g.) was refluxed with toluene-*p*-sulphonyl chloride (7 g.) for 2 hr. The dark brown precipitate was dissolved in 0·1N-sodium hydroxide (200 c.c.), diluted to 600 c.c., refluxed with charcoal, and filtered. The filtrate was added slowly to excess of dilute sulphuric acid and recrystallization of the resulting precipitate from aqueous ethanol gave colourless needles (3 g.) of the pure *toluenesulphonamido-thiazine*, m. p. 235—236° (decomp.) (Found : N, 8·3. $C_{15}H_{14}O_3N_2S_2$ requires N, 8·4%).

6-p-*Acetamidobenzenesulphonamido*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*. *p*-Acetamidobenzenesulphonyl chloride (6 g.) was refluxed for 1 hr. with a pyridine solution (15 c.c.) of the 6-amino-derivative (4·5 g.). The product was refluxed with acetone (200—250 c.c.), then

* *J.*, 1952, 787, is considered to be Part I of this series.

filtered hot, and, on addition of water, the required *compound* was precipitated. It formed pale yellow feathery needles (5 g.), m. p. 305° (decomp.), from nitrobenzene (Found : N, 10·9. $C_{16}H_{15}O_4N_3S_2$ requires N, 11·1%).

6 : 7-*Diethoxy*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*. A solution of 2 : 3-dihydro-6 : 7-dihydroxy-3-oxobenzo-1 : 4-thiazine (5 g.) (Mackie and Raeburn, *J.*, 1952, 787) in ethanolic sodium ethyloxide (50 c.c.) was refluxed (2—3 hr.) with ethyl sulphate (12 g.) in an inert atmosphere. After cooling and dilution with water, the product was filtered off, dissolved in hot benzene (charcoal), and filtered. Light petroleum (b. p. 60—80°) precipitated the 6 : 7-*diethoxythiazine* which formed pale yellow rectangular prisms (0·3 g.), m. p. 136—137°, from aqueous ethanol (Found : C, 56·4; H, 6·1. $C_{12}H_{15}O_3NS$ requires C, 56·2; H, 6·3%).

6-*Bromo*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*. 4-Bromo-2-nitroaniline (40 g.) was diazotised and the solution added to a well-cooled mixture of mercaptoacetic acid (25 g.) and aqueous sodium acetate (containing 60 g.). A solution in aqueous sodium carbonate of the thick red oil obtained was decomposed on warming, and acidification of the aqueous solution of the product afforded a bright yellow precipitate of the crude (4-*bromo*-2-*nitrophenylthio*)*acetic acid* (19 g.), which was sufficiently pure for the reduction. It crystallised from aqueous ethanol as yellow needles, m. p. 216—217° (Found : C, 33·3; H, 2·0; N, 4·9. $C_8H_6O_4NBrS$ requires C, 32·9; H, 2·1; N, 4·8%).

Reduction of this acid gave the 6-bromo-thiazine, colourless needles (8 g.), m. p. 204—205° (from aqueous ethanol) (Found: C, 39·3; H, 2·5; N, 5·5. Calc. for C_8H_6ONBrS: C, 39·3; H, 2·5; N, 5·7%). Mackie and Raeburn (*loc. cit.*) gave the m. p. of the supposedly impure bromo-compound as 220°, with a nitrogen content of 5·0%, but there appears to be no doubt that the compound obtained by the above method is pure bromo-derivative.

(4-*Methyl*-2-*nitrophenylthio*)*acetic acid*, similarly prepared from 3-nitro-4-aminotoluene (20 g.), crystallised from aqueous ethanol as yellow needles (6 g.), m. p. 182—183° (Found : C, 47·7; H, 4·0; N, 6·2. $C_9H_9O_4NS$ requires C, 47·6; H, 4·0; N, 6·2%), and on reduction afforded the 6-*methyl-thiazine*, pale cream-coloured needles (4 g.) (from aqueous acetic acid), m. p. 178—179° (partial decomp.) (Found : C, 60·6; H, 5·1; N, 8·0. C_9H_9ONS requires C, 60·3; H, 5·0; N, 7·8%).

6-tert.-*Butyl*-2 : 3-*dihydro*-3-*oxobenzo*-1 : 4-*thiazine*.—4-*tert*.-Butyl-2-nitroaniline (10 g.) (Shoesmith and Mackie, *J.*, 1928, 2336) gave (4-tert.-*butyl*-2-*nitrophenylthio*)*acetic acid*, yellow needles (1 g.), m. p. 162—163° (from aqueous ethanol) (Found : C, 53·4; H, 5·6. $C_{12}H_{15}O_4NS$ requires C, 53·5; H, 5·6%), and thence the 6-tert.-*butyl-thiazine*, colourless plates (0·2 g.), m. p. 170—171° (from aqueous ethanol) (Found : C, 63·7; H, 6·8; N, 6·0. $C_{12}H_{15}ONS$ requires C, 65·2; H, 6·8; N, 6·3%).

The authors thank the Agricultural Research Council for a grant, Professor F. Bell for his interest, Dr. J. W. Minnis for some of the microchemical analyses, and Dr. J. Raeburn for the preparation of one of the compounds.

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PREPARATION OF PHENOTHIAZINE DERIVATIVES AS POSSIBLE ANTHELMINTICS

By

ALEXANDER MACKIE

and

A. ANTHONY CUTLER

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Preparation of Phenothiazine Derivatives as Possible Anthelmintics.

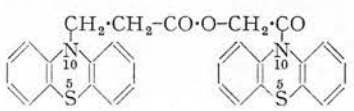
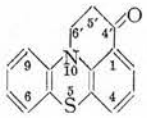
By Alexander Mackie and A. Anthony Cutler.

[Reprint Order No. 5263.]

10-Aroyl derivatives of phenothiazine and β-10-phenothiazinylpropionic acid, with salts, esters, and other derivatives have been prepared as possible anthelmintics. The acid is lethal towards liver fluke (*Fasciola hepatica*) *in vitro*.

The present paper deals with attempts to prepare improved anthelmintics of the phenothiazine type (I). First, we record that we failed to effect ring-closure, by sulphur, of 2 : 4-dinitro- and 4′-methyl-2 : 4-dinitro-diphenylamine (Reitzenstein, *J. pr. Chem.*, 1903, **68**, 256), 4′-chloro-2 : 4-dinitrodiphenylamine (Reverdin and Crépieux, *Ber.*, 1903, **36**, 33), and 2-amino-4-nitrodiphenylamine and its acetyl derivative (Nietzki and Almenräder, *ibid.*, 1895, **28**, 2971). Although a small amount of hydrogen sulphide was formed when 4-chlorodiphenylamine (Chapman, *J.*, 1929, 569) was heated with sulphur, no chlorophenothiazine could be isolated. Hydrogen bromide was evolved with the 4 : 4′-dibromodiphenylamine, but no hydrogen sulphide could be detected; possibly the sulphur removed the bromine from the compound, which would explain the presence of hydrogen bromide.

Ethyl β-10-phenothiazinylpropionate has been reported by Cauquil and Cassadevall (*Compt. rend.*, 1947, **225**, 578). The alkyl esters were more easily obtained by means of the silver salt of the acid. 10-Phenothiazinylcarbonylmethyl β-10-phenothiazinylpropionate (I) was prepared, since a compound with two phenothiazine residues might have interesting anthelmintic properties. Two arylamides of the acid were also prepared.

(I)  (II) 

Since it has been found that compounds with a keto-methylene group showed anthelmintic activity *in vitro* (Mackie and Raeburn, *Brit. J. Pharmacol.*, 1952, **7**, 219), 5′ : 6′-dihydro-4′-oxopyridino(3′ : 2′ : 1′-1 : 10*a* : 10)phenothiazine (II) and its benzylidene derivative and semicarbazone were prepared. Baldwin (*ibid.*, 1948, **3**, 91) demonstrated anthelmintic activity *in vitro* for benzylidene derivatives of ketones. The benzylidene derivative did not form a phenylhydrazone.

Although the tests of these compounds against liver fluke (*Fasciola hepatica*) and anterior preparations of the roundworm *Ascaris lumbricoides* will be reported in full elsewhere, it may be mentioned that β-10-phenothiazinylpropionic acid was lethal (1 : 3000) and its sodium salt paralysant (1 : 1000) towards liver fluke.

Experimental

10-*Benzoylphenothiazine*.—A dry benzene solution (50 c.c.) containing phenothiazine (5 g.) and benzoyl chloride (7 g.) was refluxed for 1 hr. After removal of the benzene, the residue was warmed with aqueous potassium hydroxide (20%; 40 c.c.), and the product filtered off, washed with water, and recrystallised from glacial acetic acid as yellow needles of the benzoylated compound (6 g.), m. p. 177—178° (Found: C, 74·8; H, 4·7. Calc. for $C_{19}H_{13}ONS$: C, 75·3; H, 4·3%). Fraenkel (*Ber.*, 1885, **18**, 1844) gives m. p. 170·5°.

10-(2 : 4-*Dichlorobenzoyl*)*phenothiazine*.—A xylene solution (25 c.c.) of freshly distilled 2 : 4-dichlorobenzoyl chloride (6 g.) and phenothiazine (5 g.) was refluxed for 45 min. The xylene was distilled off till the solution became turbid. The crystals obtained on cooling recrystallised from ethanol as colourless prisms of the 2 : 4-*dichlorobenzoyl* derivative (4 g.), m. p. 133—134° (Found: C, 61·5; H, 3·1. $C_{19}H_{11}ONCl_2S$ requires C, 61·3; H, 3·0%).

10-p-*Nitrobenzoylphenothiazine*.—*p*-Nitrobenzoyl chloride (5 g.) was added gradually to an acetic acid solution of phenothiazine (5 g. in 25 c.c.) and the mixture heated for 15 min. Next morning, the product was filtered off and washed in turn with ethanol and acetone. Recrystallisation from xylene afforded bright yellow rectangular plates (3·5 g.), m. p. 225—226°, of the *nitrobenzoyl* derivative (Found : C, 65·9; H, 3·5. $C_{19}H_{12}O_3N_2S$ requires C, 65·5; H, 3·5%).

10-(3 : 5-*Dinitrobenzoyl*)*phenothiazine*.—This *compound* was similarly obtained. Recrystallisation from xylene afforded yellow plates (40% yield), m. p. 265—266° (Found : C, 58·1; H, 2·9. $C_{19}H_{11}O_5N_3S$ requires C, 58·0; H, 2·8%).

10-p-*Anisoylphenothiazine*.—*p*-Anisoyl chloride (9 g.) was added gradually to phenothiazine (10 g.) and pyridine (10 c.c.). A vigorous reaction ensued on warming and the colourless crystals formed on cooling were washed with ethanol and recrystallised from glacial acetic acid. Colourless rectangular prisms of the p-*anisoyl* derivative (13 g.), m. p. 173—174°, were obtained (Found : C, 71·9; H, 4·7. $C_{20}H_{15}O_2NS$ requires C, 72·1; H, 4·2%).

β-10-*Phenothiazinylpropionic Acid*.—This compound was prepared by Smith's method (*J. Org. Chem.*, 1950, **15**, 1125). The sodium salt, obtained by titration, crystallised from absolute ethanol–acetone as colourless needles, m. p. 262—263° (decomp.). The 1-*phenylethylammonium* salt formed colourless needles, m. p. 156—158° (Found : C, 70·5; H, 5·8. $C_{23}H_{24}O_2N_2S$ requires C, 70·4; H, 6·1%), and the S-*benzylthiuronium* salt was obtained as colourless needles, m. p. 160° (both from aqueous ethanol) (Found : C, 63·1; H, 5·2. $C_{23}H_{23}O_2N_3S_2$ requires C, 63·2; H, 5·3%). The *piperazonium* salt crystallised from absolute ethanol as colourless needles, m. p. 190—191° (Found : C, 63·5; H, 5·9. $C_{34}H_{36}O_4N_4S_2,H_2O$ requires C, 63·2; H, 5·9%).

The silver salt of the acid, an alkyl iodide, and sodium-dried benzene were refluxed on the water-bath for 2 hr. After removal of the silver iodide, the filtrate was extracted with aqueous sodium hydrogen carbonate and evaporated. The residue was a red oil which solidified on cooling and was purified by recrystallisation from ethanol (charcoal). The *esters*, except the ethyl ester, are tabulated : all formed colourless needles, except the *iso*propyl (colourless rectangular plates) and the *sec.*-butyl (colourless prisms) ester. The *n*-heptyl ester became greenish-blue in light.

β-10-*Phenothiazinylpropionic esters*.

Ester	Formula	Found (%) C	Found (%) H	Required (%) C	Required (%) H	M. p.	Yield (%)
Methyl	$C_{16}H_{15}O_2NS$	68·1	5·4	67·4	5·3	64—65°	37
n-Propyl	$C_{18}H_{19}O_2NS$	69·1	6·1	69·0	6·1	34—35	42
*iso*Propyl	$C_{18}H_{19}O_2NS$	69·6	6·4	69·0	6·1	74—75	73
n-Butyl	$C_{19}H_{21}O_2NS$	70·1	6·5	69·7	6·4	85—86	74
*iso*Butyl	$C_{19}H_{21}O_2NS$	69·3	6·4	69·7	6·4	73—74	70
sec.-Butyl	$C_{19}H_{21}O_2NS$	69·5	6·4	69·7	6·4	43—44	80
tert.-Butyl	$C_{19}H_{21}O_2NS$	69·2	6·7	69·7	6·4	76	70
n-Amyl	$C_{20}H_{23}O_2NS$	70·7	6·8	70·4	6·7	74—75	68
n-Hexyl	$C_{21}H_{25}O_2NS$	70·7	7·0	70·9	7·0	52—53	36
n-Heptyl	$C_{22}H_{27}O_2NS$	71·7	7·3	71·6	7·3	46—47	70
n-Octyl	$C_{23}H_{29}O_2NS$	72·2	7·8	72·1	7·6	38—39	70

4-*Nitrobenzyl* β-10-*phenothiazinylpropionate* crystallised from xylene–light petroleum (b. p. 60—80°) in bright yellow needles (55%), m. p. 160—161° (Found : C, 64·2; H, 4·5. $C_{22}H_{18}O_4N_2S$ requires C, 65·0; H, 4·4%).

10-*Phenothiazinylcarbonylmethyl* β-10-*Phenothiazinylpropionate*.—An ethanolic solution of the sodium salt of the acid (2 g.) and 10-chloroacetylphenothiazine (Dahlbom and Ekstrand, *Acta Chem. Scand.*, 1951, **5**, 107) (2 g.) was refluxed on the water-bath for 3 hr. The product which separated afforded colourless rectangular prisms of the *ester* (1 g.), m. p. 179—180°, from toluene–light petroleum (b. p. 40—60°) (Found : C, 67·9; H, 4·1. $C_{29}H_{22}O_3N_2S_2$ requires C, 68·2; H, 4·3%).

β-10-*Phenothiazinylpropion*-p-*toluidide*.—A xylene solution (15 c.c.) of the acid (4 g.) and *p*-toluidine (8 g.) was refluxed for 3 hr. Benzene (20 c.c.) was added to the cooled product, and the benzene–xylene solution washed in turn with dilute hydrochloric acid, water, aqueous sodium hydroxide, and again water. After drying and removal of the benzene, light petroleum (b. p. 40—60°) was added to the residue. The *toluidide* which separated recrystallised from benzene–light petroleum (b. p. 40—60°) (charcoal) as colourless matted needles (3 g.), m. p. 140° (Found : C, 72·8; H, 5·6. $C_{22}H_{20}ON_2S$ requires C, 73·3; H, 5·6%).

β-10-*Phenothiazinylpropion*-p-*bromoanilide*.—This *derivative* was prepared similarly from the

acid (2 g.). Recrystallisation from absolute ethanol (charcoal) gave colourless needles (1 g.), m. p. 193—194° (Found : Br, 18·2. $C_{21}H_{17}ON_2BrS$ requires Br, 18·8%).

5′-Benzylidene-5′ : 6′-dihydro-4′-oxopyridino(3′ : 2′ : 1′-1 : 10a : 10)*phenothiazine*.—5′ : 6′-Dihydro-4′-oxopyridino(3′ : 2′ : 1′-1 : 10*a* : 10)phenothiazine (Smith, *loc. cit.*) (5 g.), benzaldehyde (5 g.), absolute ethanol (70 c.c.), and 5N-sodium hydroxide (3·5 c.c.) were shaken and set aside for 4 hr. ; the precipitated *benzylidene* derivative recrystallised from aqueous methanol in yellow prismatic needles (3·5 g.), m. p. 164° (Found : C, 77·5; H, 5·1. $C_{22}H_{15}ONS$ requires C, 77·4; H, 4·4%).

5′ : 6′-Dihydro-4′-oxopyridino(3′ : 2′ : 1′-1 : 10a : 10)*phenothiazine Semicarbazone*.—The *semicarbazone* crystallised from chlorobenzene–light petroleum (b. p. 40—60°) as yellow needles, m. p. 237—238° (Found : C, 62·7; H, 4·7; N, 17·6. $C_{16}H_{14}ON_4S$ requires C, 61·9; H, 4·5; N, 18·1%).

The authors are indebted to the Agricultural Research Council for a grant and to Dr. J. W. Minnis for some of the microchemical analyses.

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PREPARATION OF RHODANINE DERIVATIVES AS POSSIBLE ANTHELMINTICS

By

ALEXANDER MACKIE

and

ANAND L. MISRA

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November, 1954, (5525), pages 3919—3922.

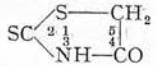
Preparation of Rhodanine Derivatives as Possible Anthelmintics.

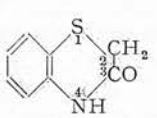
By Alexander Mackie and Anand L. Misra.

[Reprint Order No. 5525.]

Rhodanine derivatives, including metallic derivatives of rhodanine, benzylidenerhodanines, compounds containing phenothiazine residues, and phenylimino-compounds have been prepared as possible anthelmintics. A mechanism for the formation of the phenylimino-compounds is suggested. Some of the benzylidene derivatives are very active towards liver fluke (*Fasciola hepatica*).

Rhodanine derivatives have been prepared with the view to testing of their anthelmintic activity *in vitro* against the roundworm *Ascaris lumbricoides* and liver fluke (*Fasciola hepatica*), since rhodanine (I) contains the group $-S{\cdot}CH_2{\cdot}CO{\cdot}NH-$, as does 2 : 3-dihydro-3-oxobenzo-1 : 4-thiazine (II), which was paralysant towards liver fluke (Mackie and Raeburn, *Brit. J. Pharmacol.*, 1952, **7**, 219).

(I) 

 (II)

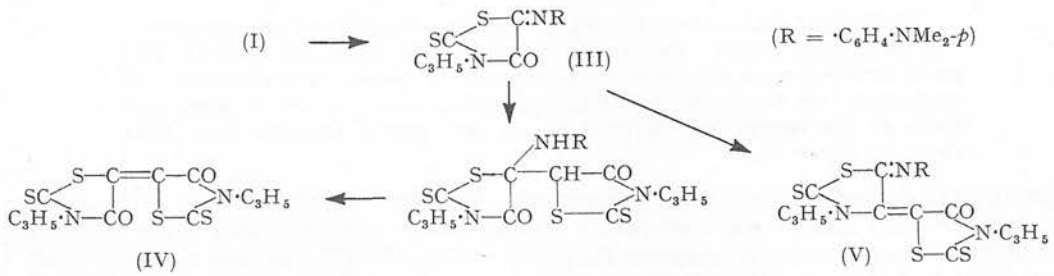
Cupric, silver, and mercuric derivatives of rhodanine were prepared by precipitation methods (cf. Nencki, *J. pr. Chem.*, 1877, **16**, 4) and benzylidene derivatives by refluxing rhodanine with aldehydes and sodium acetate in glacial acetic acid (Campbell and McKail, *J.*, 1948, 1253). A number have already been reported, but were prepared by different methods, and in some cases the melting points quoted showed considerable differences. 2 : 4-Di-(2 : 4-dinitrophenyl)rhodanine was obtained in a similar manner from 2 : 4-dinitrochlorobenzene.

An attempt was made to obtain 5-benzylidene-2 : 4-dioxothiazolidine by hydrolysis, with 20% hydrochloric acid, of the bright orange-red 2-phenylhydrazone (A; m. p. 219°, sintering at 210°) from benzylidenerhodanine (Granacher, *Helv. Chim. Acta*, 1920, **3**, 152). A yellow compound (B) was isolated, which on recrystallisation from aqueous ethanol or glacial acetic acid, or on treatment with hot water, gave a dark red compound (C), m. p. 214° (no sintering; mixed m. p. with A showed no depression). Owing to the ready conversion into C, analytically pure B could not be prepared.

Infra-red spectra of A, B, and C revealed that A and C were identical, as were A, B, and C in chloroform solution. The substance B is possibly the unstable imide hydrochloride (presence of chlorine ions confirmed). The colour difference between A and C is almost certainly due to particle size.

When 3-allylrhodanine reacted with *p*-nitrosodimethylaniline in presence of fused sodium acetate and glacial acetic acid, the phenylimino-compound (III) was obtained in very small yield, together with 3 : 3′-diallyl-4 : 4′-dioxo-2 : 2′-dithio-5 : 5′-dithiazolidinylidene (IV). However, condensation in ethanol gave the asymmetric mono-*p*-dimethylaminophenylimino-compound (V). The mechanism of the condensation of *p*-nitrosodimethylaniline with compounds containing the ketomethylene group has previously been studied, *e.g.*, with β-coumaranone (Fries *et al.*, *Ber.*, 1910, **43**, 212; 1911, **44**, 114, 124) and thioindoxyl (Mann *et al.*, *J.*, 1942, 404; 1945, 893, 913). Mann found that the formation of thioindigo or thioindirubin from thioindoxyl depended on the medium and catalyst employed, strongly

acidic media favouring the formation of thioindirubin, and weakly acidic, neutral, or basic media giving thioindigo. It appears that a similar mechanism applies to the condensations with 3-allylrhodanine as indicated in the annexed scheme. The structures of (III), (IV), and (V) were substantiated by their infra-red spectra, their CO frequencies being 1764, 1695, and 1709 cm.$^{-1}$ respectively. A five-membered cyclic lactam would be expected to absorb at about 1750 cm.$^{-1}$ if not conjugated, and at about 1710 cm.$^{-1}$ if αβ-unsaturated. The value for (IV) indicates that this is conjugated and has a *trans*-configuration. Both CO groups in (IV) were identical and the spectrum indicated a symmetrical structure, since no C:C frequency was exhibited. That all compounds contain the vinyl group is shown by the following wave-numbers: (III) 935, 985, 1420 cm.$^{-1}$; (IV) 925, 985, 1420 cm.$^{-1}$; (V) 990, 1385 cm.$^{-1}$; and *p*-substituted aromatic rings in (III) and (V) are indicated by the values 830—817 and 813 cm.$^{-1}$ respectively.



As phenothiazine is an anthelmintic, rhodanine and some of its derivatives were condensed with phenothiazine derivatives.

Unsuccessful attempts were made to condense rhodanine with γ-oxo-10-phenothiazinylbutyric acid (Winnick and Faith, U.S.P. 2,461,460/1949), according to the method of Allan, Maclean, and Newbold (*J.*, 1952, 5053).

3-Allyl-5-hydroxyiminorhodanine was prepared by the action of sodium nitrite on 3-allylrhodanine in aqueous acetic acid, with the view to obtaining the diketone and subsequently quinoxalines, but the hydroxyimino-compound could not be hydrolysed.

The biological tests will be published elsewhere; some of the benzylidene derivatives were very active towards liver fluke.

Experimental

A slight excess of aqueous copper sulphate, silver nitrate, and mercuric acetate was added severally to ethanolic solutions of rhodanine for the cupric and mercuric salts and to an aqueous ammoniacal solution of rhodanine for the silver salt. The precipitates were washed in turn with water and hot ethanol. Rhodanine (1·25 g.) gave a copper salt (2 g.), dark brown amorphous powder, m. p. 128—130° (decomp.) (Found: C, 21·5; H, 1·4. Calc. for $C_6H_4O_2N_2S_4Cu$: C, 21·9; H, 1·2%), a silver salt (2·5 g.), greenish-yellow amorphous powder, m. p. 230—232° (decomp.) (Found: C, 15·7; H, 1·2. Calc. for $C_3H_2ONS_2Ag$: C, 15·0; H, 0·8%), and a mercuric salt (2·2 g.), yellow amorphous powder, m. p. 240° (decomp.) (Found: C, 14·3; H, 0·9. Calc. for $C_6H_4O_2N_2S_4Hg,H_2O$: C, 14·7; H, 1·2%).

3-*Allylrhodanine*.—This was obtained (68%) by Andreasch and Zipser's method (*Monatsh.*, 1903, **24**, 499). It had b. p. 139°/6 mm., m. p. 48—49°. Andreasch and Zipser obtained a yellow oil.

Benzylidene Derivatives.—These were obtained by Campbell and McKail's method (*J.*, 1948, 1253), as follows: *o*-Nitrobenzylidenerhodanine, yellow needles (from glacial acetic acid) (83%), m. p. 188—190° (decomp.); Bondzynski (*Monatsh.*, 1887, **8**, 349) gives m. p. 188—190° (decomp.). Salicylidenerhodanine, yellow needles (from glacial acetic acid) (63%), m. p. 218—220° (decomp.); Zipser (*ibid.*, 1902, **23**, 958) gives m. p. 200° (decomp.); Bargellini (*Atti R. Accad. Lincei*, 1906, **15**, [i], 35) gives 218—219° (decomp.). *p*-Hydroxybenzylidenerhodanine, yellow needles (from aqueous pyridine) (75%), m. p. 258—260° (decomp.); Bargellini (*loc. cit.*) gives m. p. 260°. *p*-Anisylidenerhodanine, yellowish-brown needles (from glacial acetic acid) (77%), m. p. 250° (decomp.), sinters at 240° (Found: C, 53·3; H, 3·6. Calc. for $C_{11}H_9O_2NS_2$: C, 52·6; H, 3·6%); Andreasch and Zipser (*loc. cit.*) give m. p. 130—142° (decomp.).

Cinnamylidenerhodanine, yellow needles (from xylene) (81%), m. p. 220—225° (decomp.); Andreasch and Zipser (*Monatsh.*, 1902, **23**, 958) give m. p. 208—211° (decomp.); Bargellini (*loc. cit.*) gives 220—221° (decomp.). *o*-Nitrocinnamylidenerhodanine, yellow needles (from aqueous pyridine) (72%), m. p. 248—250°; Brown, Bradsher, Bond, and Potter (*J. Amer. Chem. Soc.*, 1951, **73**, 2357) give m. p. 250°. Piperonylidenerhodanine, yellow needles (from aqueous pyridine) (48%), m. p. 255° (decomp.); Andreasch and Zipser (*loc. cit.*, 1903) give m. p. 245° (decomp.); Bargellini (*loc. cit.*) gives 256—258° (decomp.). *p*-Dimethylaminobenzylidenerhodanine, deep red needles (from xylene) (56%), m. p. 270° (decomp.); Andreasch and Zipser (*Monatsh.*, 1905, **26**, 1191) give m. p. 246° (decomp.); Bargellini (*Atti R. Accad. Lincei*, 1906, **15**, [i], 181) gives 270° (decomp.). Furfurylidenerhodanine, golden-yellow needles (from absolute ethanol) (87%), m. p. 229—231° (decomp.) (Found: C, 45·4; H, 2·6. Calc. for $C_8H_5O_2NS_2$: C, 45·4; H, 2·4%). Andreasch and Zipser record sintering at 204° only; Bargellini (*loc. cit.*, p. 35) gives m. p. 220° (decomp.). 3-Formyl-10-methylphenothiazine (Buu-Hoï and Hoán, *J.*, 1951, 1834) (4·25 g.) gave 5-(10-*methyl*-3-*phenothiazinylmethylene*)*rhodanine*, purified by recrystallisation from aqueous pyridine as dark red needles (5 g.), m. p. 247—248° (decomp.) (Found: C, 57·3; H, 3·8. $C_{17}H_{12}ON_2S_3$ requires C, 57·3; H, 3·4%). 3-*Allyl*-5-(10-*methyl*-3-*phenothazinylmethylene*)*rhodanine* was obtained similarly from 3-allylrhodanine as brick-red prisms (82%) (from aqueous acetone), m. p. 162—164° (Found: C, 60·5; H, 4·3. $C_{20}H_{16}ON_2S_3$ requires C, 60·6; H, 4·0%). 10-(5-*Benzylidene*-4-*oxo*-2-*thiazolinylthioacetyl*)*phenothazine* was prepared by adding to hot ethanolic benzylidenerhodanine (Andreasch and Zipser, *loc. cit.*) (2 g. in 125 c.c.) aqueous sodium acetate (4 g. in 5 c.c.), followed by boiling ethanolic 10-chloroacetylphenothiazine (Dahlbom and Ekstrand, *Acta Chem. Scand.*, 1951, **5**, 102) (3 g. in 10 c.c.). The reddish mixture was refluxed on the water-bath for 4 hr. and the product which separated recrystallised from glacial acetic acid as pale yellow needles (1·75 g.), m. p. 214—216° (decomp.) (Found: C, 62·1; H, 3·1. $C_{24}H_{16}O_2N_2S_3$ requires C, 62·6; H, 3·5%). The p-*chlorobenzylidene* analogue was similarly obtained from *p*-chlorobenzylidenerhodanine (McKail and Campbell, *J.*, 1948, 1253) (2·5 g.) and 10-chloroacetylphenothiazine (3 g.), forming pale yellow needles (2·5 g.), m. p. 213—214°, from glacial acetic acid (Found: C, 58·6; H, 3·3. $C_{24}H_{15}O_2N_2S_3Cl$ requires C, 58·2; H, 3·0%).

5-*Benzylidene*-4-*oxo*-2-*phenylhydrazonothiazolidine*.—This compound (A) was prepared by Granacher's method (*Helv. Chim. Acta*, 1920, **3**, 152). It recrystallised from xylene in bright orange-red plates, m. p. 219°, sintering at 210°. It (1·5 g.) was refluxed with 20% hydrochloric acid (100 c.c.) for 3 hr., the colour changing to pale yellow. The suspension was filtered. The residue was washed with cold water and ether and dried *in vacuo*. When this yellow compound (B) (1·5 g.) was heated at about 170°, or recrystallised from aqueous ethanol or glacial acetic acid, or treated with hot water, a dark red compound (C) was obtained, m. p. 214° (no sintering); the mixed m. p. with A showed no depression, but the m. p. of C was not raised by repeated recrystallisation.

4-(2 : 4-*Dinitrophenyl*)-2-(2 : 4-*dinitrophenylthio*)-4-*oxothiazolidine*.—Hot aqueous sodium acetate (3·5 g. in 5 c.c.) was added to a boiling absolute ethanolic solution of rhodanine (1 g. in 10 c.c.), then hot absolute ethanolic 2 : 4-dinitrochlorobenzene (3 g. in 10 c.c.). The mixture was refluxed for 2 hr. The *product* which separated recrystallised from aqueous acetone as yellow prisms (2 g.), m. p. 197—198° (Found: N, 15·5. $C_{15}H_7O_9N_5S_2$ requires N, 15·1%).

3-*Allyl*-5-p-*dimethylaminophenyliminorhodanine* (III).—3-Allylrhodanine (8·5 g.), *p*-nitrosodimethylaniline (7·5 g.), fused sodium acetate (10 g.), and glacial acetic acid (30 c.c.) were refluxed for 4 hr. The *product* separated as a gum which crystallised from absolute ethanol as red needles (1 g.), m. p. 155—156°, sinter at 140° (Found: N, 13·3. $C_{14}H_{15}ON_3S_2$ requires N, 13·7%).

3 : 3′-*Diallyl*-4 : 4′-*dioxo*-2 : 2′-*dithio*-5 : 5′-*dithiazolylidene* (IV).—The gummy mass obtained as above was refluxed with benzene (charcoal). The *product* crystallised from benzene–ethanol as orange plates (1·5 g.), m. p. 184—186° (Found: C, 42·5; H, 2·7; N, 7·5. $C_{12}H_{10}O_2N_2S_4$ requires C, 42·1; H, 2·9; N, 8·1%).

3 : 3′-*Diallyl*-5-p-*dimethylaminophenylimino*-4′-*oxo*-2 : 2′-*dithio*-4 : 5′-*dithiazolidinylidene* (V).—The above condensation, carried out in ethanol instead of acetic acid, was complete in 0·5 hr. The *product* which separated recrystallised from acetic acid (charcoal) and then from alcohol as dark red needles (1·25 g. from 3·5 g.), m. p. 145—146° (Found: C, 51·9; H, 4·3; N, 12·9. $C_{20}H_{20}ON_4S_4$ requires C, 52·2; H, 4·3; N, 12·2%). Its red ethanolic solution changed to violet in presence of a trace of silver, being thus distinguished from (III) and (IV) which gave no colour.

3-*Allyl*-5-*hydroxyiminorhodanine*.—Aqueous sodium nitrite (6·5 g. in 15 c.c.) was added slowly to a warm suspension of 3-allylrhodanine (15 g.) in 2N-acetic acid (150 c.c.), and the

mixture slightly warmed for 15 min. The yellow oil changed to reddish-orange. The supernatant liquid was decanted and on cooling deposited the *hydroxyimino*-compound. The oil was repeatedly extracted with boiling water and the supernatant liquid cooled as before. Recrystallisation from benzene afforded the pure compound as yellow plates (5 g.), m. p. 147—148° (Found: N, 13·6. $C_6H_6O_2N_2S_2$ requires N, 13·9%).

We thank the Agricultural Research Council for financial assistance and the Council of Scientific and Industrial Research (India) for the award (to A. L. M.) of an Assam Oil Co. Scholarship, Dr. L. J. Bellamy, Ministry of Supply, Chemical Inspectorate, for the infra-red spectral determinations and their interpretation, and Dr. J. W. Minnis for some of the microchemical analyses.

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PREPARATION OF THIAZOLES AND BENZOTHIAZOLES AS POSSIBLE ANTHELMINTICS

By

ALEXANDER MACKIE

and

ANAND L. MISRA

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PAPER No. 11

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Preparation of Thiazoles and Benzothiazoles as Possible Anthelmintics.

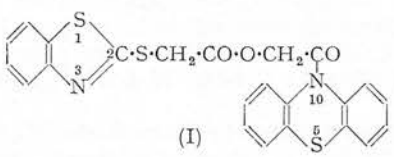
By ALEXANDER MACKIE and ANAND L. MISRA.

[Reprint Order No. 5594.]

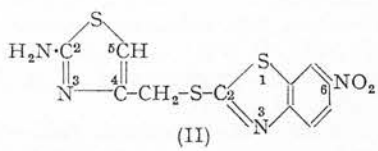
Derivatives of mercaptobenzothiazoles, benzothiazoles, and thiazoles have been prepared, most containing phenothiazine residues. Some of the compounds were very active against liver fluke (*Fasciola hepatica*) *in vitro*.

2-MERCAPTOBENZOTHIAZOLE has been found to be lethal towards liver fluke (*Fasciola hepatica*) and to be strongly depressant against the roundworm *Ascaris lumbricoides*, both *in vitro*. On account of this and the structural relation to the anthelmintics 2 : 3-dihydro-3-oxobenzo-1 : 4-thiazine, rhodanine (Mackie and Raeburn, *Brit. J. Pharmacol.*, 1952, **7**, 219; Mackie and Misra, *J.*, 1954, 3919), and phenothiazine, further thiazole derivatives have been prepared.

Compounds were prepared by condensing mercaptobenzothiazoles with 10-chloroacetylphenothiazine or its ω-pyridinium derivative. The *S*-benzylthiuronium and piperazinium salts of (benzothiazolylthio)-acetic and -propionic acid, and the 10-phenothiazinylcarbonylmethyl ester (I) of the former acid were obtained. A sulphone was prepared by condensing 2-methyl-6-benzothiazolesulphonyl chloride with phenothiazine in pyridine. Also derivatives of 2-aminothiazole, such as (II), were prepared. Ziegler (*J. Amer. Chem. Soc.*, 1941, **63**, 2946; 1944, **66**, 744) found that *p*-acetamidobenzenesulphonyl chloride did not react with 2-amino-4-(α-ethoxycarbonylalkyl)thiazoles and concluded that 4-carboxy- or 4-ethoxycarbonyl groups exerted an inhibitory effect on the 2-amino-group of the thiazole in this respect; the compound (II) did not react with toluene-*p*-sulphonyl chloride in pyridine and an analogous reason can be suggested. Although the ease of removal of a 2-halogen atom in benzothiazole was influenced by a nitro-group in the 6-position, to which electrophilic substitution is usually directed (Colonna, *Pubbl. Inst. Chim. Univ. Bologna*, 1943, No. 2—7, 3; *Chem. Abs.*, 1947, **41**, 754), 2-chloro-6-nitrobenzothiazole (Katz, *J. Amer. Chem. Soc.*, 1951, **73**, 4007) did not condense with phenothiazine (cf. Gilman and Shirley, *ibid.*, 1944, **66**, 825, 888; Cauquil and Cassadevall, *Compt. rend.*, 1947, **225**, 578).



(I)



(II)

Details of the biological testing will be reported elsewhere, but we may mention the high activity towards liver fluke *in vitro* of some of the compounds, especially 2-mercapto-6-nitrobenzothiazole, which is paralysant at 1 : 80,000.

EXPERIMENTAL

(10-*Acetyl*-2-*phenothiazinylthio*)*benzothiazole*.—A saturated ethanolic solution of 10-chloroacetylphenothiazine, m. p. 118—119° (5·5 g.) (Ekstrand, *Acta Chem. Scand.*, 1949, **3**, 302, gives m. p. 115—116·5°), was shaken with a mixture of a saturated ethanolic solution of mercaptobenzothiazole (3·5 g.) and aqueous sodium acetate (3·5 g. in 5 c.c.). Reaction set in immediately on warming on the water-bath; after 1 hour's refluxing the *product* which separated was recrystallised from benzene as buff prisms (7 g.), m. p. 168—169° (Found: C, 62·9; H, 3·8. $C_{21}H_{14}ON_2S_3$ requires C, 62·1; H, 3·5%).

2-*Mercapto*-6-*nitrobenzothiazole*.—To concentrated sulphuric acid (145 c.c.) was added gradually mercaptobenzothiazole (33 g.), followed by potassium nitrate (22 g.) portionwise, at $<10°$ with stirring. After 1 hr. the product was poured into water, a mixture of the 6-nitro-derivative and 6 : 6′-dinitrodibenzothiazole disulphide separating. This dissolved when refluxed with aqueous sodium sulphide for 20 min., and the solution was filtered and acidified with dilute acetic acid; the 6-nitro-derivative was precipitated and recrystallised from glacial acetic acid in yellow needles (30 g.), m. p. 255—256°. Teppema and Sebrell (*J. Amer. Chem. Soc.*, 1927, **49**, 1779) gave m. p. 255—256°, and Drozdov and Stavroskaya (*J. Gen. Chem. U.S.S.R.*, 1937, **7**, 2313) m. p. 226°.

Similarly, 2-(10-*acetylphenothiazinylthio*)-6-*nitrobenzothiazole* was prepared from 2-mercapto-6-nitrobenzothiazole (2 g.) as pale yellow plates (from benzene) (2 g.), m. p. 222—224° (Found : C, 56·5; H, 3·0. $C_{21}H_{13}O_3N_3S_3$ requires C, 55·9; H, 2·9%).

N-(10-*Phenothiazinylcarbonylmethyl*)*pyridinium Salts*.—Dry pyridine (5 g.) was added to a boiling absolute ethanolic solution (35 c.c.) of 10-chloroacetylphenothiazine (11 g.) and the mixture refluxed for 8 hr. The separated pyridinium chloride was filtered off hot and purified by refluxing with absolute ethanol; it formed colourless prisms (10 g.), m. p. 252—253°. Dahlbom (Swed. P. 136,720/1952) gives m. p. 252—253°.

This pyridinium compound (3 g.) in water (15 c.c.) was added gradually to aqueous-ethanolic sodium mercaptobenzothiazole (pH 6—7; from 1 g. of the mercapto-compound) at 0—5°. The product was chilled in ice (1 hr.), then filtered off, washed with water, and recrystallised from acetone–light petroleum (b. p. 40—60°), forming yellow, shining prisms of the *salt* (2 g.), m. p. 96—98° (vigorous decomp. at 100°), of the pyridinium and thiol derivatives (Found : C, 64·1; H, 4·4. $C_{26}H_{19}ON_3S_3$ requires C, 64·3; H, 3·9%).

The *salt* from 2-mercapto-6-nitrobenzothiazole (1·5 g.) and the pyridinium compound (2·7 g.) was obtained similarly. It was purified by dissolving it in cold acetone, adding ethyl acetate to turbidity, and storage in ice overnight. (The compound decomposed in hot acetone.) It was obtained as yellow prisms (1·2 g.), m. p. 132—134° (decomp.), becoming red at 120° (Found : C, 58·1; H, 3·8. $C_{26}H_{18}O_3N_4S_3$ requires C, 58·9; H, 3·4%). The impure sample decomposed readily at 100°.

10-*Phenothiazinylcarbonylmethyl* (2-*Benzothiazolylthio*)*acetate*.—2-Mercaptobenzothiazolylacetic acid (Kucherov, *J. Gen. Chem. U.S.S.R.*, 1949, **19**, 752) gave the S-*benzylthiuronium* salt as colourless prismatic needles, m. p. 167—168°, from aqueous ethanol (Found : C, 52·5; H, 4·1. $C_{17}H_{17}O_2N_3S_3$ requires C, 52·1; H, 4·3%), and the *piperazinium* salt in colourless shining plates, m. p. 184°, from ethanol (Found : C, 48·2; H, 4·2. $C_{22}H_{24}O_4N_4S_4,H_2O$ requires C, 47·7; H, 4·7%).

A boiling saturated ethanolic solution of 10-iodoacetylphenothiazine (5 g.) (Dahlbom and Ekstrand, *Acta Chem. Scand.*, 1949, **3**, 302) was refluxed with a saturated ethanolic sodium hydroxide solution of the acid (3 g.; pH 6—7) on the water-bath for 2 hr., and the crystalline precipitate was recrystallised from benzene as colourless, shining prisms (3·5 g.) of the required *ester* (I), m. p. 148—150° (Found : C, 59·7; H, 3·3. $C_{23}H_{16}O_3N_2S_3$ requires C, 59·5; H, 3·4%).

An attempt to prepare the corresponding propionate gave only a resin.

β-(2-*Benzothiazolylthio*)*propionic Acid*.—This acid (Gribbens, U.S.P. 2,416,052/1947) gave a S-*benzylthiuronium* salt, colourless shining plates (from aqueous ethanol), m. p. 147—148° (Found : C, 53·5; H, 4·2. $C_{18}H_{19}O_2N_3S_3$ requires C, 53·3; H, 4·7%), and the *piperazinium* salt, colourless prisms (from ethanol), m. p. 168—169° (Found : C, 51·6; H, 4·9. $C_{24}H_{28}O_4N_4S_4$ requires C, 51·1; H, 5·0%).

2-*Methylbenzothiazole*-6-*sulphonic Acid*.—This acid was obtained by the method of Kiprianov, Ushenko, and Sych (*J. Gen. Chem. U.S.S.R.*, 1945, **15**, 200) in colourless shining plates (from water), m. p. $>310°$ (Found : C, 39·1; H, 3·5. Calc. for $C_8H_7O_3NS_2,H_2O$: C, 38·9, H, 3·6%). Kiprianov *et al.* give m. p. 295°. The S-*benzylthiuronium* salt crystallised from aqueous ethanol as colourless plates, m. p. 149—150° (Found : C, 46·9; H, 4·2. $C_{16}H_{17}O_3N_3S_3,H_2O$ requires C, 46·5; H, 4·6%).

2-*Methyl*-6-*benzothiazolyl* 10-*Phenothiazinyl Sulphone*.—2-Methylbenzothiazole-6-sulphonyl chloride (4·5 g.) (*idem, loc. cit.*) was added to a suspension of phenothiazine (3·5 g.) in pyridine (3 c.c.). On gentle warming a vigorous reaction ensued. The mixture was kept overnight, ethanol (5 c.c.) added, and the separated *sulphone* recrystallised from aqueous acetone (charcoal), forming colourless, prismatic needles (2·5 g.), m. p. 190—191° (Found : C, 58·5; H, 3·7. $C_{20}H_{14}O_2N_2S_3$ requires C, 58·5; H, 3·4%).

β-(2-*Amino*-4-*thiazolylmethylthio*)*propionic Acid*.—2-Amino-4-thiazolylmethylthiuronium hydrochloride (5 g.) (Sprague, Land, and Ziegler, *J. Amer. Chem. Soc.*, 1946, **68**, 2155), β-chloro-

propionic acid (2·5 g.), and aqueous sodium hydroxide (3·5 g. in 30 c.c.) were refluxed on the water-bath for 2·5 hr. Dilute hydrochloric acid was cautiously added to pH ~4. The precipitate, when recrystallised from water, gave the pure *acid* as light brown needles (2·5 g.), m. p. 177—178° (Found : C, 38·3; H, 4·6. $C_7H_{10}O_2N_2S_2$ requires C, 38·5; H, 4·6%).

2-*Amino*-4-(6-*nitro*-2-*benzothiazolylthio*)*methylthiazole*.—A hot aqueous-ethanolic solution (10 c.c.) of 2-amino-4-chloromethylthiazole hydrochloride (*idem*, *loc. cit.*) (1·5 g.) was refluxed with an ethanolic sodium hydroxide (1 g.) solution of 2-mercapto-6-nitrobenzothiazole (1·5 g. in 30 c.c.) for 2 hr., and the product which separated was filtered off, washed with ethanol, and recrystallised from dilute acetic acid. The *methylthiazole* was obtained as yellowish-brown, prismatic needles (1·5 g.), m. p. 230—231° (decomp.) (Found : C, 41·2; H, 2·7. $C_{11}H_8O_2N_4S_3$ requires C, 40·7; H, 2·5%). The *picrate* formed yellow needles, m. p. 210—211° (Found : N, 17·7. $C_{17}H_{11}O_9N_7S_3$ requires N, 17·7%), from acetone.

The authors thank the Agricultural Research Council for financial aid, the Council of Scientific and Industrial Research (India) for the award of an Assam Oil Co. Scholarship to one of them (A. L. M.), and Dr. J. W. Minnis for some of the microchemical analyses.

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THE PREPARATION OF SOME HETEROCYCLIC SULPHUR COMPOUNDS AS POSSIBLE ANTHELMINTICS

By

ALEXANDER MACKIE

and

ANAND L. MISRA

Journal of the Chemical Society

(Proof received)

(Reprint Order No. 5709)

PAPER No. 12

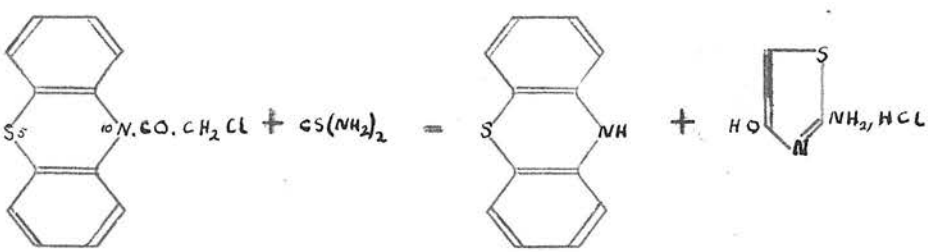
The Preparation of Some Heterocyclic Sulphur Compounds as Possible Anthelmintics.

By Alexander Mackie and Anand L. Misra

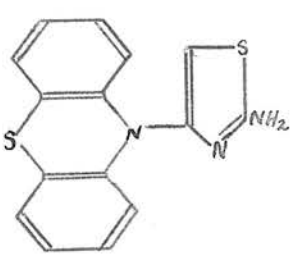
(Reprint Order No. 5709.)

The following observations were made during attempts to prepare a variety of heterocyclic sulphur compounds for use as anthelmintics.

10-Chloroacetylphenothiazine (I) was treated with thiourea with the view to obtain 2-amino-4-10'-phenothiazinylthiazole (II). Phenothiazine (III) and 2-amino-4-hydroxythiazole hydrochloride (IV) were isolated. It is possible that the hydrochloride of (II) was first formed, and was then immediately hydrolysed by ethanolic hydrochloric acid to (III) and (IV).



(I) (III) (IV)



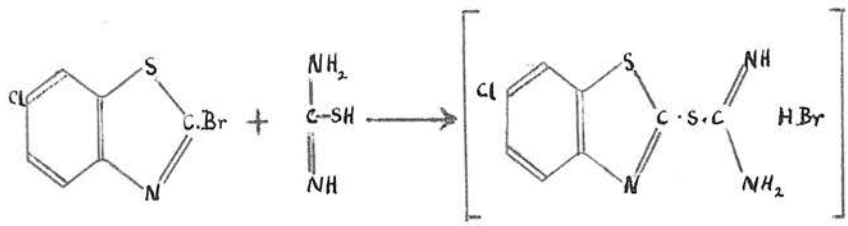
(II)

(Cf. /

(cf. Maly, Ber., 1877, 10, 1853; Davies, Maclaren, and Wilkinson, J., 1950, 3493; Ziegler, J. Amer. Chem. Soc., 1941, 63, 2946; Sprague, Land, and Ziegler, ibid., 1946, 68, 2155). When the order of addition of the reactants was reversed, a small quantity of 10-acetylphenothiazine was isolated in addition to (III) and (IV).

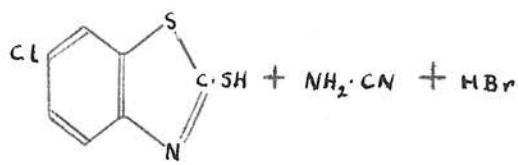
Next, 2-bromo-6-chlorobenzothiazole with thiourea was found to give 6-chloro-2-mercaptobenzothiazole (V) in good yield in ethanol and di-(6-chloro-2-benzothiazolyl) sulphide (VI) in very poor yield in water. This affords a good method for the preparation of 6-substituted 2-mercaptobenzothiazoles, which are otherwise obtained laboriously from 6-amino-2-mercaptobenzothiazole. These observations substantiate the previous findings of Scott and Watt (J. Org. Chem., 1937, 2, 148) and Watt (ibid., 1939, 4, 436). According to the mechanism postulated by these authors, the formation of (V) presumably depends on the formation of an unstable intermediate addition compound (VII), which decomposes into 6-chloro-2-mercaptobenzothiazole in ethanol, and reacts in water, owing to greater ionisation, with an additional molecule of 2-bromo-6-chlorobenzothiazole to form (VI), according to the annexed scheme.

(VII)/

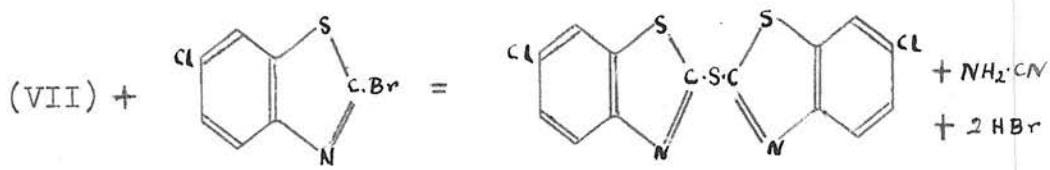


(VII)

EtOH

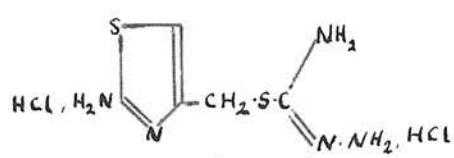


(V)



(VI)

Finally, 2-amino-4-chloromethylthiazole hydrochloride condensed with thiosemicarbazide to form an S-substituted thiosemicarbazide (VIII) (cf. Sprague, Land, and Ziegler, loc. cit.)



(VIII)

The /

The results of the biological testing of the benzothiazoles and of the thiazolyl derivatives will be published elsewhere.

Experimental. - Reaction product of 10-chloroacetylphenothiazine with thiourea. 10-Chloroacetylphenothiazine (Ekstrand, Acta Chem. Scand., 1949, 3, 302) (11.5 g.) was added to a boiling ethanolic solution (40 c.c.) of thiourea (3 g.). The mixture was refluxed for 3 hr.; plates separated, which were filtered off, washed with hot ethanol and dried (12.5 g.). Washing with cold acetone removed the phenothiazine formed in the reaction, and the residue (5 g.), on recrystallisation from methanol, gave colourless prisms of 2-amino-4-hydroxy-thiazole hydrochloride (IV) (4 g.), m.p. 206-208°(decomp.) (Found: C, 24.1; H, 3.3. Calc. for $C_3H_5ON_2ClS$: C, 23.6; H, 3.3%). When the order of the addition of the reactants in this reaction was reversed, phenothiazine and 10-acetylphenothiazine, m.p. 198 - 199°, were isolated, along with (IV).

Addition of pyridine to the hot saturated aqueous solution of the hydrochloride liberated the base, which was obtained pure on recrystallisation from water as colourless needles, becoming light brown at 220°, dark/

dark brown at 234°, m.p. 242 - 244°(decomp.) (Found: C, 30.9; H, 3.3. Calc. for $C_3H_4ON_2S$: C, 31.0; H, 3.4%); it reduced Fehling's solution.

The base has been reported by King and Miller (J. Amer. Chem. Soc., 1949, 71, 367) who give m.p. 233 - 238°(decomp.), and Davies, Maclaren, and Wilkinson (J., 1950, 3491) who give m.p. 230 - 240° (decomp.). No depression of the m.p. was observed on admixture with the tautomeric ψ-thiohydantoin, m.p. 241 - 243° (Allen and VanAllan, Org. Synth., 1947, 27, 72). The benzylidene derivative of the base obtained above sinters at 280° and has m.p. 294°(decomp.), which was not depressed when mixed with benzylidene-ψ-thiohydantoin (cf. Kucera, Monatsh., 1914, 35, 137; Stieger, ibid., 1916, 37, 653; Culvenor, Davies, Maclaren, Nelson, and Savige, J., 1949, 2573).

2-Bromo-6-chlorobenzothiazole. This compound, prepared from 2-amino-6-chlorobenzothiazole (Kaufmann and Schulz, Arch. Pharm., 1935, 273, 22) (20 g.) by Elderfield and Short's method (J. Org. Chem., 1953, 18, 1092) for the preparation of 2-bromo-4-chlorobenzothiazole, formed dark yellow needles (16.5 g.) (from ethanol), m.p. 97 - 98° (Found: C, 33.8, H, 1.1. $C_7H_3NClBrS$ requires C, 33.8; H, 1.2%).

6-/

6-Chloro-2-mercaptobenzothiazole (V). 2-Bromo-6-chlorobenzothiazole (2.5 g.) was added to a hot saturated ethanolic solution of thiourea (0.75 g.). The red solution was refluxed for 4 hr. on the water-bath. The separated product was collected and recrystallised from aqueous ethanol in clusters of pale yellow needles (1.5 g.), m.p. 250 - 252° (Found: C, 41.6; H, 2.0. Calc. for $C_7H_4NClS_2$: C, 41.7; H, 2.0%). Teppema and Sebrell (J. Amer. Chem. Soc., 1927, 49, 1779) give m.p. (not sharp) 245°, and Drozdov and Stavrovskaya (J. Gen. Chem. U.S.S.R., 1937, 7, 2813) m.p. 244 - 245°. The preparation by Sandmeyer reaction from 6-amino-2-mercaptobenzothiazole is reported in both papers.

Di-(6-chloro-2-benzothiazolyl)sulphide (VI). 2-Bromo-6-chlorobenzothiazole (2.5 g.) was added to a boiling aqueous solution of thiourea (0.75 g. in 15 c.c.), and the mixture refluxed on the water-bath for 4 hr. The pale yellow solid, which separated, was filtered off, shaken with cold 10% aqueous sodium hydroxide (150 c.c.), and collected. The residue was washed with water and recrystallised from chlorobenzene-light petroleum (b.p. 40 - 60°) as light brown needles of the sulphide (0.3 g.), m.p. 174 - 176° (Found: C, 45.9; H, 2.0. N,/

N, 7.4; S, 25.6. $C_{14}H_6N_2Cl_2S_3$ requires C, 45.5; H, 1.6; N, 7.6; S, 26.0%). 2-Bromo-6-chlorobenzothiazole (1.5 g.) was recovered.

S-(2-Amino-4-thiazolylmethyl)thiosemicarbazide dihydrochloride (VIII). 2-Amino-4-chloromethylthiazole hydrochloride (Sprague, Land, and Ziegler, J. Amer. Chem. Soc., 1946, 68, 2155) (5 g.) was added to a boiling 70 - 80% ethanolic solution (30 c.c.) of thiosemicarbazide (2.5 g.) and the mixture refluxed for 2 hr. on the water-bath. A vigorous reaction set in when complete dissolution was effected and a crystalline precipitate separated, which was collected after cooling and recrystallised from aqueous methanol in colourless prisms of the pure substituted thiosemicarbazide dihydrochloride (4.5 g.), m.p. 211 - 212° (vigorous decomp.), becoming light brown at 195° (Found: C, 21.8; H, 3.9; N, 26.2. $C_5H_{11}N_5Cl_2S_2$ requires C, 21.7; H, 4.0; N, 25.4%). The base, liberated from an aqueous solution of the hydrochloride on addition of slight excess of potassium acetate or pyridine, crystallised from water in colourless plates, m.p. 173 - 174°(decomp.).

p-Dimethylaminobenzaldehyde S-(2-amino-4-thiazolylmethyl)thiosemicarbazone. A hot ethanolic solution of p-dimethylaminobenzaldehyde (0.3 g. in 5 c.c.) was/

was added to a hot aqueous ethanolic solution (20 c.c.) of the above thiosemicarbazide hydrochloride (0.3 g.) and sodium acetate (0.35 g.). The mixture was refluxed on the water-bath for 2 hr. The separated product was cooled, collected, and recrystallised from absolute ethanol as greyish prismatic needles of the thiosemicarbazone (0.25 g.), m.p. 207 - 208°(decomp.) (Found: C, 50.3; H, 5.2. $C_{14}H_{18}N_6S_2$ requires C, 50.3; H, 5.4%).

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PREPARATION OF PHENOTHIAZINE DERIVATIVES AS POSSIBLE ANTHELMINTICS PART II

By

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and

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Journal of the Chemical Society

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PAPER No. 13

Preparation of Phenothiazine Derivatives as Possible Anthelmintics. Part II.*

By Alexander Mackie and Anand L. Misra.

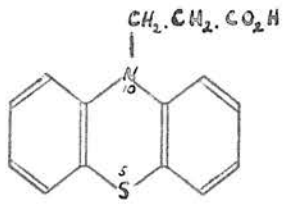
(Reprint Order No. 5845)

Since β-10-phenothiazinylpropionic acid (I) showed distinct anthelmintic activity towards liver fluke *in vitro* (Mackie and Cutler, Part I)*, it appeared desirable to prepare some derivatives of this acid, and also related compounds containing a ketomethylene group, which had been shown to induce anthelmintic activity *in vitro* (Mackie and Raeburn, Brit. J. Pharmacol., 1952, 7, 219).

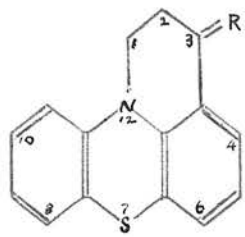
Derivatives of 2:3-dihydro-3-oxo-1H-pyrid[3,2,1-kl]-phenothiazine (IIa) (Smith, J. Org. Chem., 1950, 15, 1125), e.g., (IIb), (IIc), and (IId), have been prepared.

(I)/

* Part I, J., 1954, 2577.

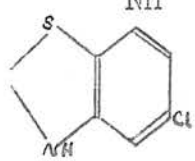


(I)



(IIa), R = :O

(IIb), R = :N.NH.C.NH.NO_2 (NH)

(IIc), R = 

(IId), R = :N.NH.CO.CH_2-N^+-Cl^- (phenyl)

In an attempt to prepare β-10-phenothiazinyl-propionyl chloride, for the purpose of lengthening the chain (Arndt-Eistert method), only the ketone (IIa) could be obtained by the action of phosphorus oxychloride on the acid (I) or its sodium salt, or of phosphorus trichloride on the acid. Thionyl chloride gave an uncrystallisable gum. Reaction of the methyl ester with aqueous ammonia over a prolonged period or fusion of the acid (I) with urea (Cherbuliez and Landolt, Helv. Chim. Acta, 1946, 29, 1438) did not produce the amide. Phenothiazine was isolated in the latter/

latter reaction. Phenothiazine was also obtained instead of the substituted benziminazole when the acid (I) was treated with o-phenylenediamine.

Bromination of the acid (I) in glacial acetic acid and acetic anhydride gave the monobromo-derivative of the ketone (IIa), whilst similar bromination of the ketone (IIa) appeared to give a difficultly separable mixture of its mono- and di-bromo-derivatives.

A quaternary hexamine salt was isolated from the reaction in chloroform of 10-chloroacetylphenothiazine (Dahlbom and Ekstrand, Acta Chem. Scand., 1951, 5, 102) with hexamine, recrystallisation of which resulted in decomposition to give hexamine hydrochloride as the only product isolated. Since it was considered that the introduction of the diethylaminoethylamino-group into a phenothiazine derivative containing a keto-methylene group might yield a compound showing anthelmintic properties, (cf. Mauss et al. Naturwiss., 1946, 33, 253; Ber., 1948, 81, 19), the chloro-compound was treated with 2-diethylaminoethylamine to form 10—2'-diethylaminoethylaminoacetylphenothiazine.

For examination of the effect of a long continuous chain on in vitro anthelmintic activity,

10-/

10-phenothiazinylcarbonylmethyl stearate $C_{12}H_8SN.CO.CH_2.O.CO.[CH_2]_{16}.Me$ was prepared by condensing 10-iodoacetylphenothiazine (Dahlbom, Acta Chem. Scand., 1953, 7, 873) with sodium stearate.

Gilman and Nelson (J. Amer.Chem. Soc., 1953, 75, 5424) obtained 10-phenylacetylphenothiazine by condensing phenothiazine with phenylacetyl chloride in dioxan. When glacial acetic acid was used as the condensing medium, 10-acetylphenothiazine was isolated in good yield with the liberation of hydrogen chloride. 10-Phenylacetylphenothiazine gave phenothiazine and not 10-acetylphenothiazine when refluxed in glacial acetic acid saturated with hydrogen chloride. Chloroacetylation of phenothiazine was effected in dry benzene (Ekstrand, Acta Chem. Scand., 1949, 3, 302) and in dioxan(Gilman and Nelson, loc. cit.), but when glacial acetic acid was used as solvent 10-acetyl-phenothiazine resulted. It appears that phenylacetyl chloride and chloroacetyl chloride react in presence of glacial acetic acid to give the mixed anhydrides $CH_2Ph.CO.O.COMe$ and $CH_2Cl.CO.O.COMe$ respectively, which in turn react with the hydrogen chloride liberated, to give in each case acetyl chloride (Watson and Gregory, J., 1929, 1373). This would explain the formation of acetyl-/

acetylphenothiazine in these reactions, since phenothiazine is readily acetylated with acetyl chloride in glacial acetic acid. Condensation of phenothiazine with some aroyl chlorides, however, proceeds smoothly in glacial acetic acid (Part I).

Experimental. - 2:3-Dihydro-3-(nitroamidino-hydrazono)-1H-pyrid-[3,2,1-kl]phenothiazine (IIb). 2:3-Dihydro-3-oxo-1H-pyrid-[3,2,1-kl]phenothiazine (IIa) (Smith, J. Org. Chem., 1950, 15, 1125) (4 g.) was added to a boiling glacial acetic acid solution of aminonitroguanidine (4 g. in 50 c.c.) (Phillips and Williams, J. Amer. Chem. Soc., 1928, 50, 2465) (cf. Whitmore, Revukas, and Smith, ibid., 1935, 57, 706). The mixture was heated for 0.5 hr., a yellow crystalline precipitate being formed. After cooling and filtration the residue was washed in turn with hot ethanol and ether, and dried. Recrystallisation from pyridine afforded golden yellow plates of the hydrazono-derivative (2.5 g.), m.p. 234 - 235° (decomp.) (Found: C, 54.3: H, 4.0. $C_{16}H_{14}O_2N_6S$ requires C, 54.2: H, 4.0%).

5'-Chloro-2:3-dihydro-1H-pyrid[3,2,1-kl]pheno-thiazine-3-spiro-2'-benzothiazoline (IIc). A pyridine solution of (IIa) (2.6 g. in 5 c.c.) was added to

2-/

2-amino-4-chlorothiophenol hydrochloride in pyridine (Lankelma and Knauff, J. Amer. Chem. Soc., 1931, 53, 310) (2 g. in 5 c.c.). The mixture was refluxed on the water-bath for 8 hr., and after cooling, acidified with dilute hydrochloric acid. The supernatant liquid was decanted, and the red gum which remained extracted in turn with ethanol and boiling light petroleum (b.p. 60-80°). The solid residue crystallised from acetone as scarlet shining needles of the spiro-compound (0.8 g.); m.p. 206 - 207° (Found: C, 64.0; H, 3.2. $C_{21}H_{15}N_2ClS_2$ requires C, 63.9; H, 3.8%). (cf. Lankelma and Sharnoff, ibid., 1932, 54, 379).

2:3-Dihydro-3-(pyridinioacetylhydrazono)-1H-pyrid-[3,2,1-kl]phenothiazine chloride (IId). A suspension of the ketone (IIa) (1.25 g.) and Girard reagent P (1 g.) in absolute ethanol (15 c.c.) containing 10% acetic acid was refluxed for 3 hr. The product which separated was filtered off, washed with hot absolute ethanol and recrystallised from chloro-benzene-light petroleum (b.p. 40 - 60°) as yellow prisms (0.3 g.), m.p. 299 - 300° (Found: C, 70.6; H, 4.0; N, 10.3%). No definite structure could be assigned to this compound. Crystals separated from the/

the filtrate after storage in ice overnight. These were recrystallised from ethanol-methanol, the pyridinioacetylhydrazono-compound being obtained as yellow stellate needles (0.7 g.), m.p. 192 - 194° (Found: N, 13.0. $C_{22}H_{19}ON_4ClS$ requires N, 13.3%).

Bromination of β-10-phenothiazinylpropionic acid. Bromine (2.9 g.) in glacial acetic acid (5 c.c.) was added to β-10-phenothiazinylpropionic acid (I) (Smith, loc. cit.) (5 g.) dissolved in a mixture of glacial acetic acid (30 c.c.) and acetic anhydride (10 c.c.). The temperature was kept at 40 - 50°. The mixture was then refluxed for 2 hr. on the water-bath; a yellow solid separated, which was collected and recrystallised from absolute ethanol. Dull yellow microscopic needles of ?-bromo-2:3-dihydro-3-oxo-1H-pyrid-[3,2,1-kl]phenothiazine (1.5 g.), m.p. 247 - 248°, insoluble in aqueous sodium carbonate, were obtained (Found: C, 54.6; H, 2.9. $C_{15}H_{10}ONBrS$ requires C, 54.2; H, 3.0%).

10-(Hexaminioacetyl)phenothiazine chloride. A mixture of 10-chloroacetylphenothiazine (Dahlbom and Ekstrand, Acta Chem. Scand., 1951, 5, 102) (3 g.) and hexamine (1.5 g.) in dry chloroform (15 c.c.) was refluxed on the water-bath for 5 hr. The colourless needles of the quaternary salt formed were filtered off/

off, washed with chloroform, refluxed for 0.5 hr. with benzene, collected hot, and dried (3.3 g.), m.p. 172 - 173° (Found: C, 57.8; H, 5.6. $C_{20}H_{22}ON_5ClS$ requires C, 57.8; H, 5.3%). Recrystallisation of the quaternary salt from ethyl acetate-absolute ethanol afforded colourless prismatic needles of hexamine hydrochloride, m.p. 204 - 205° (decomp.) (Found: C, 40.9; H, 7.0. Calc. for $C_6H_{12}N_4$,HCl: C, 40.8; H, 7.4%). Locquin (Bull. Soc. chim., 1900, 23, 663) gives m.p. 188 - 189°(decomp.).

10-(2-Diethylaminoethylaminoacetyl)phenothiazine. A mixture of 2-diethylaminoethylamine (6 g.) and 10-chloroacetylphenothiazine (5.5 g.) in dry benzene (30 c.c.) was refluxed on the water-bath for 10 hr. The resulting solution was shaken with N-hydrochloric acid, and the acid extract made alkaline with aqueous sodium carbonate. The base, which separated as an oil, solidified and was filtered off and dried. Recrystallisation from light petroleum (b.p. 40 - 80°) afforded colourless prisms of the pure base (2 g.), m.p. 79 - 80° (Found: N, 11.1. $C_{20}H_{25}ON_3S$ requires N, 11.8%) (cf. Dahlbom and Ekstrand, loc. cit.).

10-Phenothiazinylcarbonylmethyl stearate. A hot ethanolic solution of 10-iodoacetylphenothiazine (1.7 g. in 20 c.c.) (Dahlbom, Acta Chem. Scand., 1953, 7, 873) was added to an aqueous ethanolic solution of sodium stearate (1.1 g. in 15 c.c.), made slightly acid to litmus. The mixture was refluxed on the water-bath for 3 hr. On cooling, the product was filtered off and recrystallised from ethanol in colourless plates of the stearate (1.6 g.), m.p. 73 - 74° (Found: C, 73.5; H, 8.2 $C_{32}H_{45}O_3NS$ requires C, 73.4: H, 8.6%).

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IN VITRO TESTS OF CHEMICAL COMPOUNDS ON ASCARIS LUMBRICOIDES AND FASCIOLA HEPATICA

By

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IN VITRO TESTS OF CHEMICAL COMPOUNDS ON ASCARIS LUMBRICOIDES AND FASCIOLA HEPATICA

by

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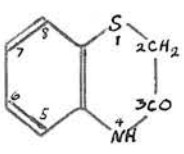
Baldwin (1948) tested over 200 chemical compounds *in vitro* against *Ascaris lumbricoides* ("roundworm") and found that anthelmintic potency was influenced by the presence of certain groups, and by the arrangement of groups within the molecule. Mackie and Raeburn (1952a) found that 2:3-dihydro-3-ketobenzo-1:4-thiazine (I) and a number of its derivatives produced a paralysant effect on *Fasciola hepatica* (liver fluke) *in vitro*, and were able to arrange substituent groups in order of potency. Azo-dyestuffs from 6-amino-2:3-dihydro-3-ketobenzo-1:4-thiazine were either inactive or only depressant, when tested *in vitro* against liver fluke/

fluke and the anterior preparations of roundworm (Mackie and Cutler, 1952).

The present paper describes an extension of this work, to include the <u>in vitro</u> testing against roundworm and liver fluke, not only of additional derivatives of (I), but also of derivatives of phenothiazine (II), rhodanine (III), and a number of miscellaneous compounds.



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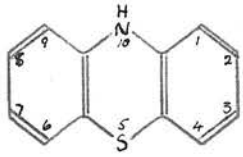

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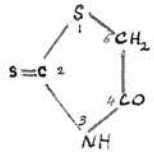


(III)

Derivatives of phenothiazine were tested because it has been so successful as an anthelmintic in veterinary practice, despite certain disadvantages. Compound (III), which contains the $-S-CH_2-CO-NH-$ group (cf. I), might be expected to show some activity, and the testing of its derivatives seemed desirable. The miscellaneous compounds tested included those which were promising against the free-living stages of sclerostomes (Parnell and Mackie, 1952).

From/

From a study of these various compounds, some observations on *in vitro* anthelmintic effect and chemical constitution have been made.

Methods

Preparative. - Many of the compounds were prepared by known methods, but the 2:3-dihydro-3-ketobenzo-1:4-thiazine derivatives and most of the phenothiazine and rhodanine derivatives were either new compounds or prepared by improved methods (Mackie and Raeburn, 1952b; Mackie and Cutler, 1953, 1954; Mackie and Misra, 1954).

Biological Testing. - The compounds were tested *in vitro* employing Baldwin's kymographic technique for roundworms (1943) and Chance and Mansour's modification of this method for liver flukes (1949). Anterior preparations of roundworms were used, since they contain the so-called "nerve-ring" (cf. Baldwin, 1943). Certain details of procedure have already been recorded (Mackie and Raeburn, 1952a).

Results/

Results

2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives. - Mackie and Raeburn (1952b) stated that this compound, and the derivatives described, had practically no effect on intermediate preparations of the roundworm. Nineteen of the 37 compounds of this type, described in the paper cited, and by Mackie and Cutler (1952, 1953), however, showed depressant effects on anterior preparations. Table I summarizes the effects of the new compounds active against roundworm or liver fluke, or both (for previous results see Mackie and Raeburn, 1952a; Mackie and Cutler, 1952). In this and other tables, all concentrations are 1:1,000, except for those compounds with paralysant (P) and lethal (L) effects, where the figures in parentheses are minimum effective concentrations. Other effects are indicated as follows: strongly depressant ++ ; depressant + ; little or no effect -.

Table I/

Table I

In vitro effect of 2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives on Ascaris lumbricoides and on Fasciola hepatica

(P = paralysant)

Substituent	Effect on	
	Ascaris	Fasciola
6-Chloroacetamido-	-	P(1:3,000)
6-Benzoylamido-	-	+
6-Toluene-p-sulphonamido-	-	+
6-p-Acetamidobenzene-sulphonamido-	-	+
6:7-Diethoxy-	-	P(1:1,000)
6-Bromo-	-	P(1:20,000) +(1:100,000)
6-Methyl-	++	P(1:5,000)
6-Tert.-butyl	-	+
Benzo-1:4-thiazine	P(1:2,000)	P(1:1,000)

The/

The derivatives already investigated by Mackie and Raeburn (1952a), had the following effects against Ascaris:

Strongly depressant: 6-acetamido-; 6-chloro-; 6-iodo-; 6-triazo-; 6-nitroso-.

Depressant: unsubstituted; 6-amino-; 6-nitro-; 6:7-dimethoxy-.

Little or no effect: 6-amino- hydrochloride; 6-fluoro-; 6-thiocyano-; 6-mercapto-; 6-arsonic acid; 6-stibonic acid; 6-chloromercuri-; 6:7-dihydroxy-; bis-(2:3-dihydro-3-ketobenzo-1:4-thiazin-6-yl).

Phenothiazine Derivatives. - The effect of phenothiazone, thionol, and phenothiazine sulphoxide, on the liver fluke and roundworm has already been described (Mackie and Raeburn, 1952c; Mackie, 1953). Since the 10-aminoacetylphenothiazines possess interesting pharmacological properties (Dahlbom and Ekstrand, 1951), some of these and a number of other phenothiazine derivatives were tested against roundworm and liver fluke in vitro. The results are recorded in Table II, except for the salts and esters of β-10-phenothiazinylpropionic acid and compounds weakly active or inactive.

Table II/

Table II

In vitro effect of phenothiazine derivatives on Ascaris lumbricoides and Fasciola hepatica

(P = paralysant; L = lethal)

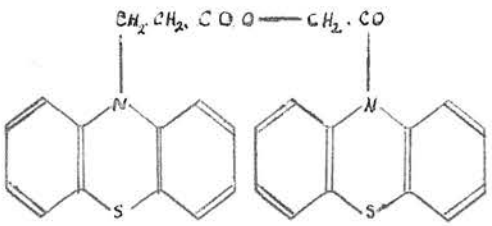
Compound	Effect on	
	Ascaris	Fasciola
Lauth's violet	-	+
Methylene blue	-	L(1:2,000) P(1:3,000)
3:7-Dinitro-10-acetyl-phenothiazine sulphoxide	+	-
10-Dimethylaminoacetyl-phenothiazine	+	-
10-Ethylaminoacetyl-phenothiazine	+	P(1:2,000)
10-Diethylaminoacetyl-phenothiazine	++	P(1:1,000)
β-10-Phenothiazinyl-propionic acid	-	L(1:3,000)
γ-10-Phenothiazinyl-γ-ketobutyric acid	-	++
5':6'-Dihydro-4'-ketopyridino-(3':2':1'-1:10a:10)phenothiazine semicarbazone	+	-

The /

The sodium, α-phenylethylammonium, S-benzylisothiuronium, and piperazinium salts of β-10-phenothiazinylpropionic acid were paralysant towards the liver fluke at 1:1,000, but only the sodium salt had any depressant action against the roundworm. Of the esters (normal from methyl to octyl; isopropyl; iso-, sec.-, and tert.-butyl; p-nitrobenzyl, and 10-phenothiazinylcarbonylmethyl; formula IV) only the isobutyl ester was active: it was strongly depressant towards roundworm and depressant towards liver fluke. The latter helminth was strongly depressed by the isopropyl ester, but the sec.- and tert.-butyl esters produced stimulant effects.

The following phenothiazine derivatives had little or no effect on either helminth: 1:3:7:9(?)-tetrachloro-; 10-methyl-; 3-formyl-10-methyl-; 10-acetyl-; 10-chloroacetyl-; 10-phenylacetyl-; 10-benzoyl-; 10-(2':4'-dichlorobenzoyl)-; 10-(4'-nitrobenzoyl)-; 10-(3':5'-dinitrobenzoyl)-; 10-anisoyl; 10-(toluene-p-sulphonyl)-; 10-(p-acetamidobenzenesulphonyl)-; 10-piperidylacetyl-; 10-morpholinylacetyl-phenothiazines; β-10-phenothiazinylpropionitrile; β-10-phenothiazinylpropion-p-toluidide; β-10-phenothiazinylpropion-p-bromoanilide;/

bromoanilide; 5':6'-dihydro-4'-ketopyridino-(3':2':1'-1:10a:10)phenothiazine (V), and its benzylidene derivative.



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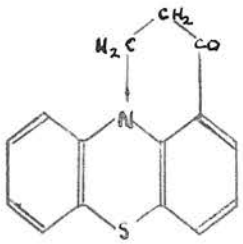

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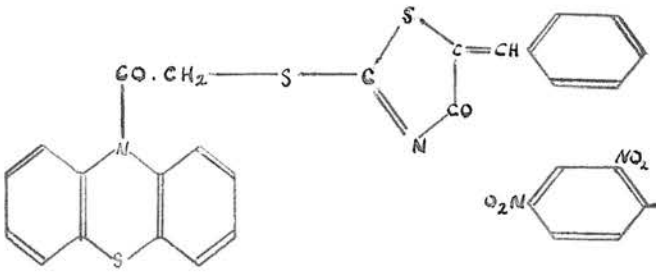

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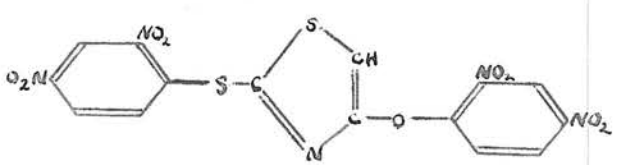

(VII)

Rhodanine Derivatives. - The results of the tests are summarized in Table III.

Table III /

Table III

In vitro effect of rhodanine derivatives on Ascaris lumbricoides and Fasciola hepatica

Compound	Effect on	
	Ascaris	Fasciola
Rhodanine	+	P(1:1,000)
3-Allylrhodanine	++	P(1:1,000)
5-isoNitroso-3-allyl-rhodanine	P(1:1,000)	L(1:2,000) P(1:4,000)
Benzylidenerhodanine	-	L(1:10,000)
Benzylidene-3-allyl-rhodanine	+	-
o-Chlorobenzylidene-rhodanine	-	L(1:2,000)
p-Chlorobenzylidene-rhodanine	-	P(1:1,000)
o-Nitrobenzylidene-rhodanine	++	L(1:2,000) P(1:3,000)
Salicylidenerhodanine	+	L(1:16,000)
p-Hydroxybenzylidene-rhodanine	-	L(1:1,000) P(1:4,000)
Anisylidenerhodanine	-	++
Cinnamylidenerhodanine	-	++
o-Nitrocinnamylidene-rhodanine	-	++
Furfurylidenerhodanine	-	L(1:3,000) P(1:4,000)
10-Methylphenothiazine-3-formylidenerhodanine	+	+
Quinrhodine	++	++

The/

The following rhodanine derivatives had little or no effect on either helminth:- cupric, silver, and mercuric rhodanides; 3-allylrhodanyl-5-p-dimethylaminoanil; 4:5'-dehydro-4:5'-bis-3-allylrhodanyl-5-p-dimethylaminoanil; 5:5'-dehydro-5:5'-bis-3-allylrhodanine; benzylidenerhodanine 2-phenylhydrazone; piperonylidenerhodanine; p-dimethylaminobenzylidenerhodanine; 10-methylphenothiazine-3-formylidene-3-allylrhodanine; S-(10-phenothiazinylcarbonylmethyl)-5'-benzylidenerhodanine (VI); S-(10-phenothiazinylcarbonylmethyl)-5'-(p-chlorobenzylidene)rhodanine; 2:4-di-(2':4'-dinitrophenyl)-rhodanine (formula VII).

Miscellaneous Compounds. - A number of miscellaneous compounds, such as aliphatic and aromatic halogen compounds, allyl compounds, mercury compounds, aromatic amines, phenols, pyridines, etc., were tested in vitro against both helminths. Some of the compounds had shown promise as sclerostome larvicides (Parnell and Mackie, 1952).

Of the halogenated compounds tested, carbon tetrabromide was the most promising, since it was six times more lethal (1:6,000 against liver fluke) than the tetrachloride. The tetrabromide was paralysant at 1:10,000, and had about the same potency against Ascaris (paralysant at 1:2,000) as the tetrachloride.

Of/

Of the α-, β-, γ-, and δ-isomers of benzene hexachloride only the δ- was active (paralysed liver fluke at 1:12,000).

Some allyl compounds were effective, especially the iodide (roundworm paralysed at 1:5,000; liver fluke killed at 1:5,000) and the isothiocyanate (liver fluke killed at 1:2,000, paralysed at 1:8,000).

Mercuric chloride and two of its organic derivatives were lethal towards liver fluke (mercuric chloride and ethylmercuric chloride at 1:20,000; ethoxyethylmercuric chloride at 1:16,000), but only ethylmercuric chloride was paralysant towards roundworm (1:2,000).

Diphenylamine was lethal towards liver fluke at 1:20,000 and paralysant towards roundworm at 1:1,000. Its derivatives, and other amino-compounds tested, had little or no effect.

Ortho- and p-nitrophenols were active, especially the p-isomer towards liver fluke (lethal at 1:4,000; paralysant at 1:12,000). The o-isomer was more potent towards roundworm (paralysant at 1:3,000).

No lethal effects were observed on liver fluke with pyridine, the picolines, 2:6-lutidine, quinoline and isoquinoline, and only α-picoline was paralysant, whereas all paralysed the anterior preparations of roundworm/

roundworm. Pyridine and α-picoline were the most effective against the latter preparation. (cf. Baldwin, 1948).

pseudoThiohydantoin had little effect (cf. rhodanine).

Essential oil ex Artemisia maritima, containing 65% β-thujone and 16% cineol-1:8, was lethal to liver flukes at 1:2,000 and paralysed at 1:3,000.

2-Amino-5-nitrothiazole (Enheptin-T), a remedy for coccidiosis, was tested against both helminths, but had no effect.

Conessine dihydrochloride had no effect on either helminth. Janot and Cavier (1949) indicated that it might be of value in the treatment of helminthiasis, although they found that it did not kill the anterior preparation of roundworm in vitro.

Pumpkin seed extract had little or no effect against liver fluke or roundworm in vitro, although the seeds have been used against tapeworm.

Sodium azide was very effective against both roundworm (paralysant at 1:10,000) and liver fluke (paralysant at 1:4,000).

Discussion/

Discussion

2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives. - Increase in the length of the side-chain in position 6 generally decreased the anthelmintic potency towards Fasciola hepatica (cf. Mackie and Raeburn, 1952a; Mackie and Cutler, 1952). The chloroacetamido-derivative was exceptional in this respect.

The order of potency of the radicals was Br > Cl > N_3, NO > I, CH_3 > NO_2, 6:7-dimethoxy > NH_2.HCl, $ClCH_2CONH$ > unsubstituted, F > NH_2, CH_3CONH, CNS, SH, 6:7-dihydroxy, 6:7-diethoxy. The order of potency of the halogens was different from that obtained with A. lumbricoides. This was not surprising, since the two helminths belong to different phyla.

The result with benzo-1:4-thiazine was interesting since liver fluke is generally more sensitive than roundworm: nevertheless the absence of the $-CH_2-CO-$ group increased the in vitro activity against the latter preparation, but decreased the potency against the former.

Phenothiazine Derivatives. - The introduction of methyl groups into Lauth's violet conferred lethal properties towards liver fluke (cf. methylene blue, which/

which has been used as an anthelmintic). The presence of a second phenothiazine residue in (IV) does not produce any marked effect.

It was surprising that the presence of the $-CH_2-CO-$ group in (V) and its benzylidene derivative had little or no effect on either helminth (cf. Baldwin, 1948).

Rhodanine Derivatives. - The position of the substituent groups in the benzene nucleus and also any substitution in the 2 and 3 positions in the rhodanine nucleus, had a marked effect on the potency of the corresponding benzylidenerhodanines.

The presence of a phenothiazine residue in the molecule produced generally little or no effect and replacement of the $-CH_2-CO-$ group in rhodanine by a quinoline residue (quinrhodine) destroyed the paralysant effect on liver fluke.

Rhodanine had only half the potency of 2:3-dihydro-3-ketobenzo-1:4-thiazine towards liver fluke (Mackie and Raeburn, 1952a), but produced the same effect on roundworm.

Miscellaneous Compounds. - In vivo experiments with carbon tetrabromide would be desirable, not only to ascertain its anthelmintic activity, but also to study its effect on the liver, which is damaged by carbon tetrachloride. The tetrabromide had a high larvicidal potency/

potency (Parnell and Mackie, 1952).

Rico (1927) found that allyl isothiocyanate paralysed A. lumbricoides in vitro, but did not give a minimum effective concentration. It has been suggested as an anthelmintic against lungworm (Mathey, 1945). The allyl compounds were very effective larvicides, especially the iodide and isothiocyanate (Parnell and Mackie, 1952). The three mercury compounds tested had similar larvicidal properties, especially ethylmercuric chloride, which merits further investigation.

Although diphenylamine only paralysed roundworm at 1:1,000, Guthrie (1940) had found it to be effective against ascarids in dogs.

The greater potency of pyridine and its derivatives, and of quinoline and of isoquinoline on roundworm than on liver fluke is exceptional. It may be that these compounds can penetrate the cuticle comparatively easily.

Summary

1. Derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, phenothiazine, and rhodanine, and some miscellaneous compounds have been tested in vitro against Fasciola hepatica and the anterior preparation of Ascaris lumbricoides.

2./

2. 2:3-Dihydro-3-ketobenzo-1:4-thiazine derivatives showed only depressant effects, when active, towards A. lumbricoides, but paralysant effects were observed with some derivatives, particularly the 6-bromo-compound, on liver fluke. Increase in the length of the side-chain usually decreased the anthelmintic potency towards liver fluke.

3. Some of the aminoacetylphenothiazines were active against liver fluke and β-10-phenothiazinylpropionic acid was lethal.

4. 5-isoNitroso-3-allylrhodanine was the only rhodanine derivative which paralysed Ascaris, but some, especially the benzylidene compounds, were lethal to the liver fluke.

5. Amongst the miscellaneous compounds the following were very active: allyl iodide and sodium azide against Ascaris; carbon tetrabromide; benzene hexachloride; allyl iodide and isothiocyanate; mercuric chloride, ethylmercuric chloride, ethoxyethylmercuric chloride; diphenylamine; and p-nitrophenol against liver fluke.

We wish to thank the Agricultural Research Council for financial support; Professor Baldwin, who trained one of us (G.M.S.) in his kymographic technique; Messrs. /

Messrs. Norval and Crombie, Edinburgh City Abattoir, for collection of parasites.

We are indebted to Professor R. D. Haworth, F.R.S. for the sample of conessine dihydrochloride and to T. & H. Smith Ltd. for the Artemisia oil and cineol-1:8.

The assistance of Miss D. Daniels in the biological testing is gratefully acknowledged. Our thanks are also due to Dr. Parnell for his interest.

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IN VITRO TESTING OF CHEMICAL COMPOUNDS AGAINST VINEGAR EELWORM (TURBATRIX ACETI)

Attempted Correlation of Anthelmintic Effect and Chemical Constitution

By

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and

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From the Department of Chemistry,
Heriot-Watt College, Edinburgh.

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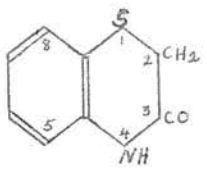
by

Alexander MACKIE and G. Marjorie STEWART

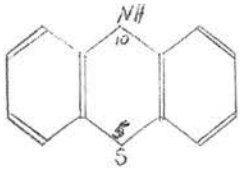
A number of chemical compounds, viz., derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine (I), of phenothiazine (II), of rhodanine (III), and miscellaneous compounds, have been tested in vitro as possible anthelmintics against liver fluke (Fasciola hepatica), and the anterior preparations of Ascaris lumbricoides, with the view to correlating if possible in vitro anthelmintic effect and chemical constitution (1, 2, 3, 4, 5).

Since some of these compounds possessed distinct in vitro anthelmintic properties, it appeared desirable to extend this work by testing these compounds, and derivatives of benzothiazole (IV) against vinegar eelworm (Turbatrix aceti), which has recently been employed for screening chemical compounds for anthelmintic/

anthelmintic activity by Leiper (6). Derivatives of (IV) were included on account of their structural relationship to (I), (II), and (III).



(I)



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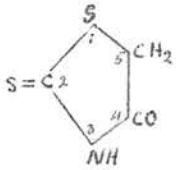

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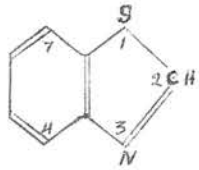


(IV)

Methods

Preparative. - Many of the compounds were prepared by known methods, but 2:3-dihydro-3-ketobenzo-1:4-thiazine, most of the phenothiazine, rhodanine, and benzothiazole derivatives were either new compounds or prepared by improved methods (7, 3, 8, 9, 10, 11, 12, 13).

Biological testing. - The method of testing the compounds against vinegar eelworm was essentially Leiper's technique (6) with certain minor modifications. As far as possible, each compound was completely dissolved in a small quantity of solvent within the toleration limits, and then added to the required volume of distilled water to give a 1:500 solution, emulsion, or suspension, which was diluted to give concentrations of 1:1,000; 1:2,000; 1:4,000; 1:8,000; 1:16,000. Equal parts (0.75 c.c.) of vinegar eelworm culture and each preparation of the compound were placed/

placed in 2 c.c. specimen tubes, so that the following concentrations of compound were actually tested:- 1:1,000; 1:2,000; 1:4,000; 1:8,000; 1:16,000; 1:32,000. The tubes were loosely corked to reduce evaporation to a minimum (the vinegar eelworm died if the tubes were tightly corked), and placed in an incubator at 36°. The times were noted at which at least 50% of the worms were killed at particular concentrations. At least five determinations were carried out on each compound at each concentration.

Results

The results of the tests are recorded for the active compounds (miscellaneous compounds excepted) in Tables I - IV. Two values are generally given for each compound. The first gives the minimum time at which at least 50% of the worms are killed by the minimum concentration of the compound. The second represents the minimum concentration required to kill at least 50% of the worms in a week. In some cases, a time less than one week is given. This indicates that the compound acts in the lesser period, but there is no activity at further dilutions.

Table I /

Table I

Effect of 2:3-dihydro-3-ketobenzo-1:4-thiazine derivatives on vinegar eelworm *in vitro*

Compound	Concentration	Time
2:3-Dihydro-3-ketobenzo-1:4-thiazine	1:2,000 1:16,000	3 hr. 1 wk.
6-Fluoro-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:1,000 1:4,000	24 hr. 1 wk.
6-Chloro-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:1,000 1:16,000	24 hr. 1 wk.
6-Iodo-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:1,000 1:16,000	24 hr. 1 wk.
6-Triazo-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:2,000 1:8,000	24 hr. 1 wk.
6-Nitroso-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:2,000 1:4,000	24 hr. 1 wk.
6-Arsonic acid 2:3-dihydro-3-ketobenzo-1:4-thiazine	1:2,000 1:8,000	24 hr. 1 wk.
6-Chloromercuri-2:3-dihydro-3-ketobenzo-1:4-thiazine	1:1,000 1:8,000	24 hr. 1 wk.
6-Amino-2:3-dihydro-3-ketobenzo-1:4-thiazine ⟶ anthranilic acid	1:1,000 1:8,000	3 hr. 1 wk.

The following derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine had little or no effect on vinegar eelworm after one week:-

6-methyl/

6-methyl, 6-tert.-butyl, 6-amino-, 6-amino-hydrochloride, 6-acetoamido-, 6-chloroacetoamido-, 6-benzoylamido-, 6-toluene-p-sulphonamido-, 6-p-acetoamidobenzenesulphonamido-, 6-bromo-, 6-thiocyano-, 6-nitro-, 6-mercapto-, 6-stibonic acid, 6:7-dihydroxy-, 6:7-dimethoxy-2:3-dihydro-3-ketobenzo-1:4-thiazines; bis-(2:3-dihydro-3-ketobenzo-1:4-thiazin-6-yl); the azo-dyestuffs 6-amino-2:3-dihydro-3-ketobenzo-1:4-thiazine ⟶ phenol, ⟶ β-naphthol, ⟶ salicylic acid, ⟶ phloroglucinol, ⟶ 4-n-hexylresorcinol, ⟶ thymol, ⟶ 2-hydroxy-3-naphthoic acid, ⟶ F-acid, ⟶ 8-hydroxyquinoline, ⟶ 1-phenyl-3-methyl-5-pyrazolone.

Table II/

Table II

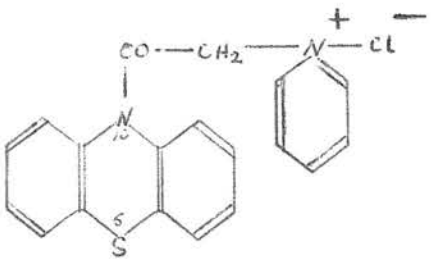
Effect of phenothiazine derivatives on vinegar eelworm *in vitro*

Compound	Concentration	Time
Phenothiazine	1:1,000	3 hr.
	1:32,000	48 hr.
Phenothiazone	1:1,000	1 hr.
	1:32,000	72 hr.
Methylene blue	1:1,000	72 hr.
	1:8,000	1 wk.
10-Chloroacetylphenothiazine	1:8,000	24 hr.
	1:8,000	1 wk.
10-Ethylaminoacetylphenothiazine	1:2,000	1 hr.
	1:8,000	2 hr.
10-Diethylaminoethylaminoacetyl-phenothiazine	1:4,000	3 hr.
	1:16,000	72 hr.
10-(Hexaminioacetyl)phenothiazine chloride	1:4,000	24 hr.
	1:4,000	1 wk.
S-Benzyl-iso-thiuronium-β-10-phenothiazinyl-propionate	1:2,000	24 hr.
	1:4,000	48 hr.
N-(10-Phenothiazinylcarbonylmethyl) pyridinium chloride (V)	1:8,000	2 hr.
	1:32,000	3 hr.

The/

The following phenothiazine derivatives had little or no effect on vinegar eelworm after one week:- thionol, phenothiazine sulphoxide; Lauth's violet; 10-methyl-, 3-formyl-10-methyl-(VI), 1:3:7:9(?)-tetrachloro-, 10-acetyl-, 10-phenylacetyl-phenothiazines; 3:7-dinitro-10-acetylphenothiazine sulphoxide; 10-benzoyl-, 10-(2:4-dichlorobenzoyl)-, 10-(4-nitrobenzoyl)-, 10-(3:5-dinitrobenzoyl)-, 10-anisoyl-, 10-(toluene-p-sulphonyl)-, 10-(p-acetoamidobenzenesulphonyl)-phenothiazines; 10-dimethylamino-, 10-diethylamino-, 10-piperidyl-, 10-morpholinyl-acetylphenothiazines; β-10-phenothiazinylpropionitrile, β-10-phenothiazinylpropionic acid, its α-phenylethylammonium and piperazinium salts, its normal esters (from ethyl to octyl), iso-propyl, iso-, sec.-, and tert.-butyl, p-nitrobenzyl and 10-phenothiazinylcarbonylmethyl esters; its p-toluidide and p-bromoanilide; γ-10-phenothiazinyl- γ-ketobutyric acid; 10-phenothiazinylcarbonylmethyl stearate; 2:3-dihydro-3-keto-1H-pyrid[3,2,1-kl]phenothiazine (VIIa), its semicarbazone, benzylidene, and nitroamidinohydrazono (VIIb) - derivatives; 2:3-dihydro-3-(pyridinioacetylhydrazono)-1H-pyrid[3,2,1-kl]-phenothiazine chloride (VIIc).

(V) /



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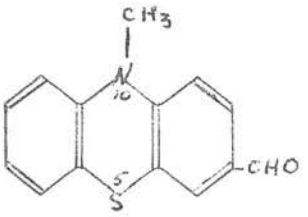

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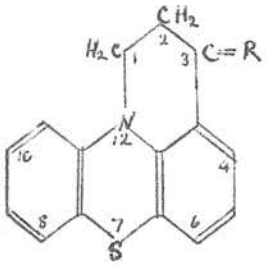


(VIIa) R = :O

(VIIb) R = :N.NH.C(=NH).NH.NO_2

(VIIc) R = :N.NH.CO.CH_2—N^+(C$_6$H$_5$)—Cl^-

Table III/

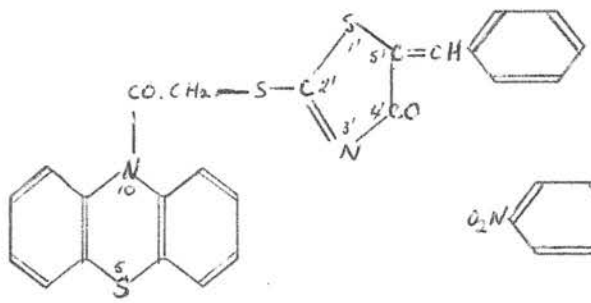
Table III

Effect of rhodanine derivatives on vinegar eelworm *in vitro*

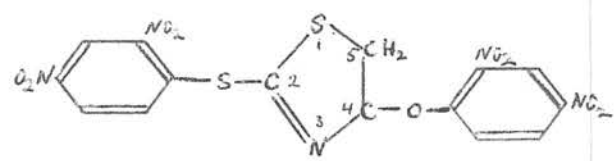
Compound	Concentration	Time
Cupric rhodanide	1:2,000	72 hr.
	1:4,000	1 wk.
Mercuric rhodanide	1:2,000	24 hr.
	1:16,000	1 wk.
3-Allylrhodanine	1:4,000	24 hr.
	1:16,000	1 wk.
5-*iso*-Nitroso-3-allylrhodanine	1:1,000	2 hr.
	1:16,000	24 hr.
3-Allylrhodanyl-5-dimethyl-aminoanil	1:1,000	1 wk.
	1:4,000	1 wk.
Cinnamylidenerhodanine	1:1,000	24 hr.
	1:32,000	1 wk.
Furfurylidenerhodanine	1:1,000	24 hr.
	1:8,000	1 wk.
10-Acetylphenothiazinyl-2'-mercapto-5'-benzyl-idenerhodanine (VIII)	1:4,000	24 hr.
	1:32,000	1 wk.
10-Acetylphenothiazinyl-2'-mercapto-5'-(p-chloro-benzylidene)rhodanine	1:1,000	24 hr.
	1:8,000	1 wk.

The/

The following rhodanine derivatives had little or no effect on vinegar eelworm after one week:- rhodanine; silver rhodanide; benzylidenerhodanine and its phenylhydrazone; benzylidene-3-allylrhodanine; o- and p- chloro-, o-nitro-, o- and p-hydroxy-, p-methoxy-benzylidenerhodanines; piperonylidene-, o-nitrocinnamylidene-, p-dimethylaminobenzylidene-, 10-methylphenothiazine-3-formylidene-, 10-methyl-phenothiazine-3-formylidene-3-allylrhodanines; 2:4-di-(2':4'-dinitrophenyl)rhodanine (IX); quinrhodine.



(VIII)



(IX)

Table IV/

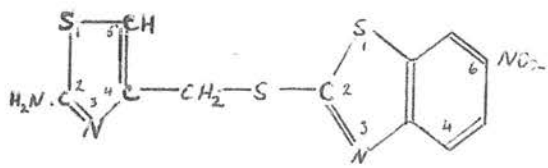
Table IV

Effect of benzothiazole derivatives on vinegar eelworm *in vitro*

Compound	Concentration	Time
2-Methylbenzothiazole	1:2,000 1:8,000	2 hr. 24 hr.
2-Chlorobenzothiazole	1:2,000 1:8,000	1 hr. 24 hr.
2-Chloro-6-nitrobenzothiazole	1:32,000	24 hr.
2-Chloro-6-aminobenzothiazole	1:1,000 1:4,000	24 hr. 1 wk.
2-Bromo-6-chlorobenzothiazole	1:4,000 1:32,000	3 hr. 24 hr.
2-Amino-4-chlorobenzothiazole	1:1,000 1:4,000	72 hr. 1 wk.
2-Amino-6-chlorobenzothiazole	1:4,000 1:8,000	24 hr. 1 wk.
2-Methylmercaptobenzothiazole	1:4,000 1:32,000	1 hr. 24 hr.
2-Mercaptobenzothiazolylacetic acid	1:1,000 1:2,000	48 hr. 72 hr.
S-Benzyl-*iso*-thiuronium salt of above	1:2,000 1:4,000	24 hr. 1 wk.
S-Benzyl-*iso*-thiuronium β-(2-mercaptobenzothiazolyl)propionate	1:1,000 1:8,000	3 hr. 1 wk.
2-(2':4'-Dinitrophenylmercapto) benzothiazole	1:1,000 1:2,000	3 hr. 24 hr.
N-(10-Phenothiazinylcarbonylmethyl)pyridinium 2-mercaptobenzothiazole	1:8,000 1:32,000	2 hr. 3 hr.

The/

The following benzothiazole derivatives had little or no effect on vinegar eelworm after one week:- 2-methylbenzothiazole-6-sulphonic acid and its S-benzyl-iso-thiuronium salt; 2-methyl-6-benzothiazolyl 10-phenothiazinyl sulphone; 2-mercaptobenzothiazole; piperazinium and 10-phenothiazinylcarbonylmethyl 2-mercaptobenzothiazolyl acetates; β-(2-mercaptobenzothiazolyl)propionic acid and its piperazinium salt; di-(2-benzothiazolyl) sulphide and disulphide; 2-(10-acetylphenothiazinylmercapto)-benzothiazole and its 6-nitro-derivative; 6-nitro-, 6-amino-, 6-chloro-mercaptobenzothiazoles; 6-nitro-2-(2':4'-dinitrophenylmercapto)benzothiazole; N-(10-phenothiazinylcarbonylmethyl)pyridinium 6-nitro-2-mercaptobenzothiazole; 2-amino-4-(6-nitro-2-benzothiazolylmercapto)methylthiazole (X); the thiazole derivative, β-(2-amino-4-thiazolylmethylmercapto)propionic acid, included for comparison.



(X)

Miscellaneous/

Miscellaneous Compounds. - About 80 miscellaneous compounds, including aliphatic and aromatic halogen compounds, allyl compounds, mercury compounds, aromatic amines, phenols, pyridines, etc., were tested in vitro against vinegar eelworms. The figures in parentheses are the minimum concentrations required to kill at least 50% of the worms within a week or less, if the compounds are ineffective at further dilutions.

The most effective of the aliphatic halogen compounds tested were n-heptyl and n-octyl iodides (1:32,000, 48 hr.); methyl iodide (1:16,000, 24 hr.); n-hexyl iodide and iodoform (1:16,000, 72 hr.); carbon tetrabromide (1:4,000, 24 hr.). None of the isomers (α-, β-, γ-, δ-) of benzene hexachloride had any effect.

The isothiocyanate (1:32,000, 1 hr.), the iodide (1:32,000, 24 hr.), and the bromide (1:4,000, 1 wk.) were the most potent of the allyl compounds tested. Allyl chloride had little effect. The three mercury compounds were very lethal, viz., ethyl mercuric chloride (1:32,000, 3 hr.); mercuric and ethoxyethyl mercuric chlorides (1:32,000, 24 hr.).

A variety of primary, secondary, and tertiary amino-compounds were examined, but only diphenylamine (1:32,000, 24 hr.) and its 4:4'-dibromo-derivative (1:32,000, 48 hr.) were of any value.

Of/

Of the phenols tested, only 4-n-hexylresorcinol (1:8,000, 3 hr.); o-nitrophenol (1:4,000, 3 hr.); thymol (1:2,000, 3 hr.) had any effect. Phenothioxin (1:32,000, 24 hr.) and xanthone (1:32,000, 1 wk.) were tested for comparison against phenothiazine.

The anthelmintics gentian violet (1:16,000, 1 wk.) and arecoline hydrobromide (1:2,000, 24 hr.) were active; but santonin and pumpkin seed extract had no effect. 2-Amino-5-nitrothiazole (Enheptin-T), a remedy for coccidiosis, and conessine dihydrochloride, suggested as a possible anthelmintic (14), had little or no effect.

Discussion

2:3-Dihydro-3-ketobenzo-1:4-thiazine derivatives. - The order of potency of the substituents in the active compounds was unsubstituted > Cl, I > N_3, H_2AsO_3 > HgCl > NO > F. Chlorine, iodine, and triazo were also the most potent substituents found with A. lumbricoides (5), and also with liver fluke (2), although in the case of the latter, this was not expected, since these two helminths belong to different phyla. The only dyestuff of interest was the diazotised 6-amino-derivative coupled with anthranilic acid.

Phenothiazine derivatives. - It is interesting to find that phenothiazine was one of the most potent of the/

the phenothiazine series. Baldwin (15) found that phenothiazine had no effect on *in vitro* preparations of *A. lumbricoides*; but the anthelmintic action of this compound still remains obscure. Phenothiazone was next in order of potency, whilst the other phenothiazine oxidation products had no effect (cf. 1, 4).

Two of the aminoacetylphenothiazines were distinctly active, especially the diethylaminoethyl-derivative, which was also the most potent of these compounds against *A. lumbricoides* (5). The diethylaminoethylamino-group is present in the Miracils (schistosomicides) (16, 17). 10-Acetyl-phenothiazine had no effect on vinegar eelworm.

Rhodanine derivatives. - 3-Allyl- and 5-*iso*-nitroso-3-allyl-rhodanines were very potent. The allyl group was an effective substituent since rhodanine had little activity. The benzylidenerhodanines were not particularly active, except the 10-acetyl-phenothiazinyl-2'-mercapto-5'-benzylidene- (VIII) (most potent of the rhodanines tested; cf. its chloro-derivative) and cinnamylidene-derivatives. Similar results were obtained with *A. lumbricoides* (5).

Benzothiazole/

Benzothiazole derivatives. - 2-Bromo-6-chloro- and 2-chloro-6-nitro- derivatives were the most potent of the benzothiazoles. The influence of groups on the *in vitro* anthelmintic properties was apparent, and position of groups in the molecule may also have determined potency (cf. 2-amino-4-chloro- and 2-amino-6-chloro-benzothiazoles). Four derivatives, lethal towards vinegar eelworm, showed paralysant effects against *A. lumbricoides* (18).

The most effective of the mercaptobenzothiazoles were the N-(10-phenothiazinylcarbonylmethyl)pyridinium (cf. the phenothiazine derivative V) and 2-methyl derivatives. The other derivatives were not outstanding in their activity. The introduction of a nitro-group in the 6-position of the pyridinium compound destroys any *in vitro* anthelmintic effect, but increases the potency of 2-chlorobenzothiazole considerably.

Miscellaneous compounds. - Although carbon tetrabromide is not the most effective of the halogen compounds tested, its activity compares favourably with its action on *A. lumbricoides* (5).

Allyl isothiocyanate was the most lethal of the miscellaneous compounds tested. The iodide was also very potent. The order of activity of the allyl compounds was N:C:S > I > Br > Cl (cf. the order with *A. lumbricoides*, I > N:C:S > Br > Cl).

The/

The mercury compounds were also very effective, particularly ethyl mercuric chloride, the only mercury compound tested, which was paralysant towards A. lumbricoides (5). The ethoxy-group apparently reduces the in vitro anthelmintic effect, as was found with A. lumbricoides.

A comparison of xanthone, phenothioxin, and phenothiazine shows that the difference in their constitution causes little change in activity.

Thirteen of the twenty-two compounds, lethal towards vinegar eelworm, also produced paralysant effects on A. lumbricoides (5).

Summary and Conclusions

1. Derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, phenothiazine, rhodanine, benzothiazole, and miscellaneous compounds have been tested in vitro against vinegar eelworm (Turbatrix aceti) at various concentrations.

2. Chemical constitution appeared to exert considerable influence on the in vitro anthelmintic activity of the compound.

3. The substituents in 2:3-dihydro-3-ketobenzo-1:4-thiazine derivatives arranged in order of potency were unsubstituted $>$ Cl, I $>$ N_3, H_2AsO_3 $>$ HgCl $>$ NO $>$ F.

4./

4. Phenothiazine and phenothiazone were the most effective in the phenothiazine series, and the introduction of the 10-diethylaminoethylamino-group into the inactive 10-acetylphenothiazine produced a considerable lethal effect.

5. Introduction of an allyl group into rhodanine increased its activity.

6. Marked influence of groups on the in vitro anthelmintic properties was found in the benzothiazoles.

7. The most lethal of the miscellaneous compounds were the following:- n-heptyl and n-octyl iodides; allyl isothiocyanate and iodide; mercuric chloride, its ethyl and ethoxyethyl derivatives; diphenylamine and its 4:4'-dibromo-derivative; phenothioxin and xanthone.

8. Many compounds, lethal towards vinegar eelworm, showed paralysant effects towards A. lumbricoides.

The authors are indebted to the Agricultural Research Council for financial assistance and to Mr. J. W. G. Leiper, M.R.C.V.S., for very kindly giving us unpublished details of his technique. Our thanks are due to Dr. I. W. Parnell for his interest in this work, and to Professor R. D. Haworth, F.R.S., for the sample of conessine dihydrochloride.

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IN VITRO TESTING OF BENZOTHIAZOLES AND SOME PHENOTHIAZINE DERIVATIVES AGAINST ASCARIS LUMBRICOIDES AND LIVER FLUKE (FASCIOLA HEPATICA)

By

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and

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From the Department of Chemistry,
Heriot-Watt College, Edinburgh.

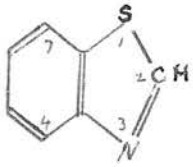
IN VITRO TESTING OF BENZOTHIAZOLES AND SOME PHENOTHIAZINE DERIVATIVES AGAINST ASCARIS LUMBRICOIDES AND LIVER FLUKE (FASCIOLA HEPATICA)

by

Alexander MACKIE, G. Marjorie STEWART,
and Anand L. MISRA

Whilst examining a number of miscellaneous compounds for in vitro anthelmintic activity, 2-mercaptobenzothiazole was found to be active against liver fluke (Fasciola hepatica) and anterior preparations of Ascaris lumbricoides. Baldwin (1) found benzothiazole (I) to be paralysant towards the latter preparations. On account of these facts and the structural relationship of (I) to 2:3-dihydro-3-ketobenzo-1:4-thiazine (II), to rhodanine (III), both paralysant towards liver fluke (2, 3), and to phenothiazine (IV), an investigation of the in vitro anthelmintic properties of the benzothiazole derivatives appeared appropriate. Some recently prepared phenothiazine derivatives were also tested.

(I)/



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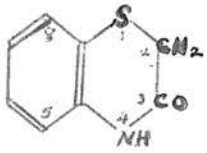

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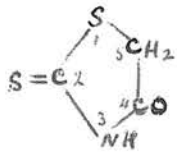

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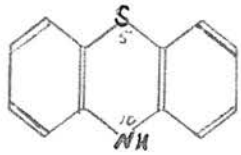


(IV)

Methods

Preparative. - Most of the compounds were either new or prepared by improved methods (4, 5, 6).

Biological. - The compounds were tested in vitro against anterior preparations of A. lumbricoides, using Baldwin's kymographic technique (7), and against liver fluke with Chance and Mansour's modification of the method (8). Other details of procedure have been recorded elsewhere (2).

Results

The results of the tests for the active compounds are summarised in Tables I and II. All the concentrations are at 1:1,000, except for those compounds with paralysant (P) and lethal (L) effects, where the figures in parentheses denote the minimum effective concentrations. Other effects are indicated as follows:- strongly depressant ++ ; depressant + ; little or no effect - .

Table I/

Table I

Effect of benzothiazole derivatives on Ascaris lumbricoides and liver fluke (Fasciola hepatica) in vitro

Compound	Effect on	
	Ascaris	Liver fluke
2-Methylbenzothiazole	P(1:1,000)	L(1:1,000)
S-Benzylthiuronium 2-methylbenzothiazole-6-sulphonic acid	+	++
2-Chlorobenzothiazole	P(1:1,000)	L(1:2,000)
2-Chloro-6-nitrobenzothiazole	++	-
2-Chloro-6-aminobenzothiazole	P(1:1,000)	++
2-Amino-4-chlorobenzothiazole	++	P(1:4,000)
2-Amino-6-chlorobenzothiazole	P(1:1,000)	L(1:3,000)
2-Mercaptobenzothiazole	++	L(1:1,000) P(1:3,000)
2-Methylmercaptobenzothiazole	-	++
6-Chloro-2-mercaptobenzothiazole	+	P(1:16,000)
6-Nitro-2-mercaptobenzothiazole	-	L(1:8,000) P(1:80,000)
2-Mercaptobenzothiazolylacetic acid	++	++
S-Benzylthiuronium salt of above	++	+
Piperazinium salt of above	-	++

10-/

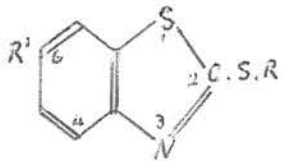
Table I (continued)

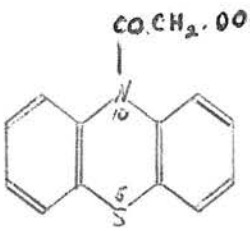
Compound	Effect on	
	Ascaris	Liver fluke
10-Phenothiazinylcarbonyl-methyl ester (Va)	+	-
β-(2-Mercaptobenzo-thiazolyl)propionic acid	-	++
S-Benzylthiuronium salt of above	-	+
Piperazinium salt of above	-	+
2-(2':4'-Dinitrophenyl-mercapto)benzothiazole	-	+
N-(10-Phenothiazinyl-carbonylmethyl)pyridinium 2-mercaptobenzothiazole (Vb)	-	P(1:1,000)
N-(10-Phenothiazinyl-carbonylmethyl)pyridinium 6-nitro-2-mercaptobenzo-thiazole (Vc)	-	P(1:16,000)

The following benzothiazole derivatives had little or no effect on either helminth:- 2-methylbenzothiazole-6-sulphonic acid; 2-methyl-6-benzothiazolyl-10-phenothiazinyl sulphone; di-(2-benzothiazolyl)sulphide and disulphide; 2-(10-acetylphenothiazinylmercapto)-benzothiazole and its 6-nitro-derivative; 2-bromo-6-chloro-, 6-amino-2-mercapto-, and 6-nitro-2-(2':4'-dinitrophenylmercapto)benzothiazoles; 2-amino-4-(6-nitro-2-benzothiazolylmercapto)methylthiazole (Vd).

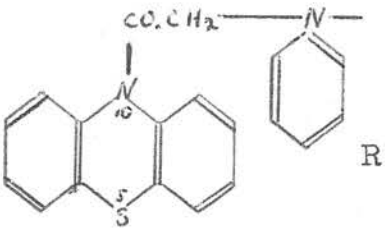
The/

The thiazole derivatives, S-(2-amino-4-thiazolylmethyl)thiuronium dihydrochloride and β-(2-amino-4-thiazolylmethylmercapto)propionic acid were tested for comparison. The latter had no effect on either helminth and the former only a depressant effect on A. lumbricoides and little action on liver fluke (cf. Baldwin 1).

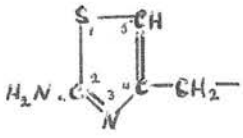


R=  R' = H

(Va)

R=  R' = H

(Vb)

R=  R' = NO_2

(Vd)

R as for (Vb) R' = NO_2

(Vc)

Table II/

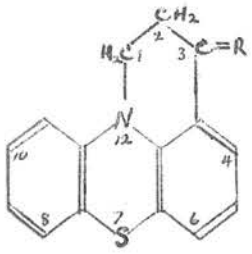
Table II

Effect of phenothiazine derivatives on Ascaris lumbricoides and liver fluke (Fasciola hepatica) in vitro

Compound	Effect on	
	Ascaris	Liver fluke
10-Diethylaminoethyl-aminoacetyl-phenothiazine	++	++
10-(Hexaminioacetyl)-phenothiazine chloride	-	L(1:2,000)
N-(10-Phenothiazinyl-carbonylmethyl)-pyridinium chloride	+	++
2:3-Dihydro-3-(nitroamidino-hydrazono)-1H-pyrid [3, 2, 1-kl] phenothiazine (VIa)	+	-
2:3-Dihydro-3-(pyridinioacetyl-hydrazono)-1H-pyrid [3, 2, 1-kl] phenothiazine chloride (VIb)	-	L(1:1,000)

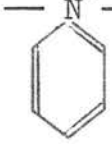
10-Phenothiazinylcarbonylmethyl stearate had little effect on both helminths.

(VIa)/



(VIa) R = :N.NH.C(=NH).NH.NO_2

(VIb) R = :N.NH.CO.CH_2 — N^+ — Cl^-



Discussion

Several benzothiazole derivatives were effective against A. lumbricoides, as well as against liver fluke. The influence of groups on the in vitro anthelmintic properties was shown and groups could be placed in order of potency against liver fluke as follows:-

2-substituted benzothiazoles. - Cl > SH > CH_3 > SCH_3

6-substituted 2-chlorobenzothiazoles. -

unsubstituted > NH_2 > NO_2

6-substituted 2-mercaptobenzothiazoles. -

NO_2 > Cl > unsubstituted > NH_2

The nitro-group decreased the activity of 2-chlorobenzothiazole, but increased the lethal and paralysant effects of 2-mercaptobenzothiazole 8 and nearly 30-fold respectively. The 6-nitro-derivative

was/

was the most potent of the 2-mercaptobenzothiazoles to be tested against liver fluke. The influence of the nitro-group was again seen in N-(10-phenothiazinyl-carbonylmethyl)pyridinium 6-nitro-2-mercaptobenzothiazole, which was 16 times more paralysant against liver fluke than the unnitrated compound (cf. 6-nitro-2:3-dihydro-3-keto-benzo-1:4-thiazine, twice as potent against liver fluke as the unsubstituted compound) (2).

Chlorine substitution in 2-mercaptobenzothiazole increased its paralysant effect more than 5 times (cf. 6-chloro-2:3-dihydro-3-ketobenzo-1:4-thiazine; 4 times more paralysant than the unsubstituted compound) (2).

The presence of another benzothiazolyl or mercapto-benzothiazolyl residue decreased the <u>in vitro</u> anthelmintic effect, as was illustrated in the lack of activity in the dibenzothiazolyl sulphide and disulphide respectively. Baldwin (1) also found a decrease in activity when a compound contained two potentially potent groups.

Position of groups in the molecule may also influence activity (cf. 2-amino-4-chloro- and 2-amino-6-chloro-benzothiazoles).

2:3-Dihydro-3-ketobenzo-6-(pyridinioacetoamido)-1:4-thiazine chloride was prepared with the view to comparing its activity with that of other pyridinioacetyl or carbonylmethyl pyridinium compounds in Tables I and/

and II. It had no effect on liver fluke and little effect on A. lumbricoides. Substitution of the mercaptobenzothiazolyl residue for the chlorine in N-(10-phenothiazinylcarbonylmethyl)pyridinium chloride induced a paralysant effect on liver fluke.

10-Phenothiazinylcarbonylmethyl stearate was prepared to ascertain whether a phenothiazine derivative with a long continuous side-chain, containing a fatty acid residue, had an effect on in vitro anthelmintic activity, since stearates and related compounds might penetrate the cuticle of A. lumbricoides (cf. 9, 10, 11).

Summary and Conclusions

1. Benzothiazoles and a few selected phenothiazine derivatives were tested against liver fluke (Fasciola hepatica) and anterior preparations of Ascaris lumbricoides in vitro.

2. Some benzothiazoles were effective against both helminth preparations, and the influence of groups and their position in the molecule on the in vitro anthelmintic properties was very marked. Several groups could be placed in order of potency against liver fluke.

3. 6-Nitro-2-mercaptobenzothiazole was the most potent of the compounds against liver fluke (lethal at 1:8,000; paralysant at 1:80,000).

The/

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AN ABNORMAL REACTION WITH GIRARD REAGENT P

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Submitted to Chemistry and Industry

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AN ABNORMAL REACTION WITH GIRARD REAGENT P

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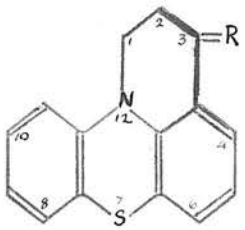
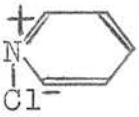
In a recent communication[1], it was reported that 2:3-dihydro-3-oxo-1H-pyrid/3,2,1-kl/phenothiazine (Ia) reacted with Girard reagent P (carbohydrazidomethylpyridinium chloride) in presence of absolute ethanol-acetic acid to give two products, A and B. Compound (A) separated from the reaction mixture in small yield and on recrystallisation from chlorobenzene-light petroleum (40 - 60°) gave yellow prisms, m.p. 299 - 300°(decomp.). These were insoluble in hot water, hot methanol, hot ethanol, hot acetone; sparingly soluble in hot chloroform and hot benzene; very soluble in hot dioxan and hot pyridine. No trace of halogen was found, and boiling with concentrated hydrochloric acid or concentrated aqueous potassium hydroxide produced no change. The filtrate from the reaction product yielded (B), the expected pyridinioacetylhydrazono-derivative (Ib), which crystallised/

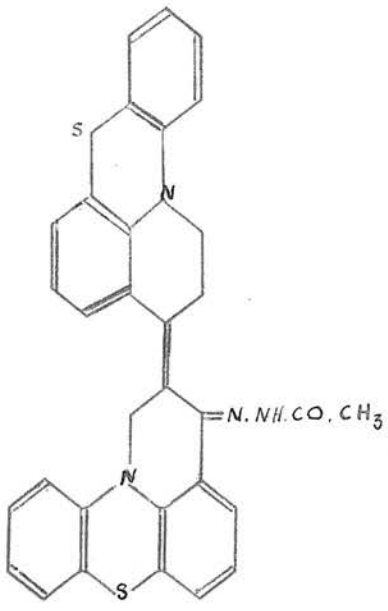
crystallised from ethanol-methanol as yellow needles, m.p. 192 - 194°, soluble in hot water, hot methanol, hot ethanol; insoluble in hot acetone and hot non-hydroxylic solvents. Hydrolysis with N. HCl gave the original ketone.



(Ia) R = :O

(Ib) R = :N.NH.CO.CH_2· 

(Ic) R = :N.NH.CO.CH_3



(II)

Compound/

Compound (A) has been further investigated. It is suggested that the compound has the structure (II) (Found: C, 70.6; H, 4.0; N, 10.3; S, 12.2. $C_{32}H_{24}ON_4S_2$ requires C, 70.6; H, 4.4; N, 10.3; S, 11.8%), and is probably formed by some of (Ib) reacting with unchanged ketone (Ia). This was substantiated by causing equimolecular quantities of (Ia) to react with (Ib) under similar conditions, when crystals separated from the reaction mixture in small yield, obtained as yellow prisms from chlorobenzene-light petroleum (40 - 60°), m.p. 300 - 303° (decomp.), not depressed when mixed with compound (A). An attempt to obtain compound (A) by treating the ketone (Ia) with acetohydrazide[2] under the same conditions as the reaction of (Ia) with Girard reagent P, afforded only the 2:3-dihydro-3-acetohydrazono-derivative (Ic), yellow needles from chlorobenzene, m.p. 258 - 259° (Found C, 65.8; H, 4.5. $C_{17}H_{15}ON_3S$ requires C, 66.0; H, 4.9%), which could be hydrolysed to the ketone (Ia) with warm concentrated hydrochloric acid.

The infra-red spectra of A and B were determined, but owing to the complexity of these molecules, interpretation/

interpretation of the spectra proved difficult. However, one absorption band, with sharp peak, at 1580 cm.$^{-1}$, in the spectrum of compound (A), but absent in that of compound (B), was very conspicuous. This corresponds to C=C stretching vibrations, and it is known that a direct linkage of an unsaturated group to the nucleus enhances the intensity of this band to such a degree, that it becomes more pronounced in the spectra, thus enabling the detection of conjugation with the rings. The proposed structure of compound (A) is in agreement with these observations. Details of the spectra are furnished in Table I.

Table I/

Table I

Prominent Infra-red Spectral Bands for Compounds (A) and (B)

Compound (A) $cm.^{-1}$	Compound (B) $cm.^{-1}$
2900 - 2845	3450
1580	2900 - 2845
1445 - 1430	1668
1410	1450 - 1422
1365	1435
1310 - 1300	1370
1268 - 1260	1210
1235	785 - 768
1218	747
785	738
750	718 - 715
732	685

We thank the Council of Scientific and Industrial Research (India) for the award (to A.L.M.) of an Assam Oil Co. Scholarship, and Dr. L. J. Bellamy, Ministry of Supply, Chemical Inspectorate, for the infra-red determinations.

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A COMPARISON OF THE RESULTS OF FOUR *IN VITRO* ANTHELMINTIC TESTING TECHNIQUES

By

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A COMPARISON of the RESULTS of FOUR *in vitro* ANTHELMINTIC TESTING TECHNIQUES.

by

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Introduction.

During the last decade the amount of interest in *in vitro* testing of compounds for anthelmintic activity has increased considerably, mainly because the need for better anthelmintics has become more and more evident, and to find them large numbers of compounds must be screened. Undoubtedly *in vitro* tests are fundamentally less satisfactory than are *in vivo* tests; however, a combination of several techniques obviates some of the objections, and allows more chemicals to be screened, on a preliminary basis, than would *in vivo* trials against a similar number of nematodes or platyhelminths. In all preliminary screening trials it is essential that the compounds should be tested for anthelmintic effect against several different helminths, because a compound may have no anthelmintic effect on some worms but be effective against others. It is also important, of course, that when compounds are tested that none with anthelmintic activity should be missed in the preliminary screening. Furthermore, *in vitro* testing of compounds for anthelmintic activity has the advantage over *in vivo* testing in that compounds which would be lethal to the host can be compared, and, therefore, that data can be obtained on chemical structures which tend to induce anthelmintic properties, which would be unobtainable from *in vivo* tests.

This/

This paper compares the results obtained by four of the best known *in vitro* techniques with fifty-two compounds; it also gives the results from some known anthelmintics when they were tested *in vitro*.

Material and Methods.

The *in vitro* techniques compared are those which use the free-living stages of Sclerostomes [1,2,3,4,5,6], (a technique which was originally designed to find compounds suitable for use against the pre-parasitic stages), sections of *Ascaris lumbricoides*[7], liver fluke (*Fasciola hepatica*)[8] and vinegar eelworms (*Turbatrix aceti*)[9]. In the technique which used the free-living stages of Sclerostomes, the compounds were added to fresh horse faeces, and, after culturing, the numbers of larvae from the treated faeces were compared with the numbers from the untreated faeces. Compounds were tested against *A. lumbricoides* and *F. hepatica* by attaching the anterior portion of the former and the whole of the latter to a kymograph, and recording the changes in their movements when the helminths were immersed in an aqueous solution, emulsion or suspension of the compound. The technique with *T. aceti* consisted of adding vinegar containing all ages of vinegar eelworms to an equal quantity of an aqueous solution, emulsion, or suspension of the compound, and observing the effect on the vitality of the eelworms.

Most/

Most of the results quoted in this paper have already been published[3,7,8,10,11,12,13,14], but they are recorded in several journals and this is the first time that the anthelmintic effect of the compounds on the different helminths has been compared.

A solution, emulsion or suspension of 1:1,000 was the most concentrated preparation used against vinegar eelworms, A.lumbricoides and liver fluke, except that two compounds were tested at concentrations of 1:500 against A.lumbricoides and liver fluke.

Results.

Table I compares the anthelmintic effect of fifty-two compounds on the free-living stages of Sclerostomes, on vinegar eelworms, on A.lumbricoides and on liver fluke.

In the column showing the effect of the compounds on Sclerostomes the minimum concentration of compound to faeces which killed over 90 per cent of the pre-parasitic stages is shown. Some compounds were most effective when applied in the dry state or undiluted with water, and other compounds were most effective when applied in dilute, or moderately concentrated, or saturated aqueous solutions. When the compound produced no lethal effect it is shown by the letter "U".

The/

The effect of the same compounds on vinegar eelworms is shown by stating the minimum concentration which killed at least 50 per cent in less than a week. The minimum concentration used was 1:32,000. When a compound at this concentration was lethal in less than a week, the time taken to kill the nematodes is shown in hours in parentheses. When over 50 per cent were still alive after a week in a 1:1,000 concentration it is indicated by a "U".

In the columns showing the effect on the anterior preparations of A. lumbricoides, the minimum concentration which had an effect in less than 30 min. is given, and the extent of this effect is indicated as follows:-

P. paralysant; S.D. strongly depressant;
D. depressant; U. little or no effect, and the most concentrated preparation which was tested is shown in brackets.

The effects of the compounds on liver fluke after 45 min. are shown in the final column of Table I. The same symbols are used and in addition "L" signifies that the compound was lethal.

Table I includes some known anthelmintics; some others have been tested by one or more of the in vitro techniques; the results are given in Table II.

Table I/

Table I.

Showing the largest number of parts of faeces or of water with which one part of the compound was mixed and an anthelmintic effect was observed on the free-living stages of Sclerostomes, on vinegar eelworms (T.aceti), on A.lumbricoides and on liver fluke (F.hepatica) in vitro.

Compound. (a).	Free-living Sclerostomes; concn. in faeces to kill 90%. (b).	Vinegar eelworms; concn. to kill 50%. (c).	A.lumbricoides; concn. (d).	A.lumbricoides; effect. (e).	Liver fluke; concn. (f).	Liver fluke; effect. (g).
Methyl iodide	50,000	16,000	1,000	P.	2,000	L.
Ethyl iodide	6,700	1,000	500	P.	500	L.
n-Propyl iodide	3,000	2,000	500	P.	1,000	L.
Methylene iodide	25,000	2,000	2,000	P.	2,000	P.
Iodoform	16,700	16,000	1,000	D.	1,000	S.D.
Carbon tetrabromide	5,000	4,000	2,000	P.	6,000 10,000	L, P.
Allyl chloride	1,000	2,000	1,000	D.	(1,000)	U.
Allyl bromide/						

Table I (continued).

(a).	(b).	(c).	(d).	(e).	(f).	(g).
Allyl bromide	14,000	4,000	1,000	P.	5,000	L.
Allyl iodide	83,000	32,000 (24)	5,000	P.	5,000	L.
Allyl isothiocyanate	100,000	32,000 (1)	4,000	P.	2,000 8,000	L, P.
Mercuric chloride	12,500	32,000 (24)	1,000	S.D.	20,000	L.
Ethyl mercuric chloride	25,000	32,000 (3)	2,000	P.	20,000	L.
Ethoxyethyl mercuric chloride	16,700	32,000 (24)	1,000	S.D.	16,000	L.
Urea	200	U.	(1,000)	U.	(1,000)	U.
Chlorobenzene	900	2,000	1,000	P.	2,000	L.
Bromobenzene	1,100	U.	1,000	P.	2,000	L.
Iodobenzene	900	2,000	1,000	S.D.	3,000	L.
o-Dichlorobenzene	900	U.	1,000	P.	1,000	L.
p-Dichlorobenzene	800	U.	1,000	D.	1,000	S.D.
Benzene/						

Table I (continued).

(a).	(b).	(c).	(d).	(e).	(f).	(g).
Benzene hexachloride α -isomer	U.	U.	(1,000)	U.	(1,000)	U.
" " β -isomer	U.	U.	(1,000)	U.	(1,000)	U.
" " γ -isomer	U.	U.	(1,000)	U.	1,000	D.
" " ξ -isomer	U.	U.	(1,000)	U.	1,000 12,000	L. P.
2:3:5:6-Tetrachloronitrobenzene	6	U.	1,000	D.	(1,000)	U.
Aniline	3,300	U.	(1,000)	U.	(1,000)	U.
Diphenylamine	8	32,000 (24)	1,000	P.	20,000	L.
o-Nitrophenol	400	4,000	3,000	P.	1,000	P.
p-Nitrophenol	1,000	U.	1,000	P.	4,000 12,000	L, P.
Thymol	100	2,000	5,000	P.	10,000	L.
4-n-Hexylresorcinol	16	8,000	10,000 to 5,000	P.	10,000 to 5,000	L.
Gentian/						

Table I (continued).

(a).	(b).	(c).	(d).	(e).	(f).	(g).
Gentian violet	80	16,000	(2,500)	U.	5,000	L.
Essential oil ex Artemisia maritima, containing 65% β-thujone and 16% cineol-1:8	1,000	U.	1,000	S.D.	2,000 3,000	L. P.
2-Mercaptobenzothiazole	3	1,000	1,000	S.D.	1,000 3,000	L. P.
Pyridine	600	U.	5,000	P.	(1,000)	U.
α-Picoline	1,400	U.	5,000	P.	1,000	P.
β-Picoline	900	U.	1,000	P.	(1,000)	U.
2:6-Lutidine	2,000	U.	2,000	P.	1,000	S.D.
Quinoline	1,100	U.	1,000	P.	1,000	S.D.
2:3-Dihydro-3-ketobenzo-1:4-thiazine	200	16,000	1,000	D.	2,000	P.
6:7-Dimethoxy-2:3-dihydro-3-ketobenzo-1:4-thiazine 6/	2	U.	1,000	D.	4,000	P.

Table I (continued).

(a).	(b).	(c).	(d).	(e).	(f).	(g).
6-Amino-2:3-dihydro-3-ketobenzo-1:4-thiazine	U.	U.	1,000	D.	1,000	P.
Hydrochloride of 6-Amino-2:3-dihydro-3-ketobenzo-1:4-thiazine	13	U.	(1,000)	U.	3,000	P.
6-Chloro-2:3-dihydro-3-ketobenzo-1:4-thiazine	U.	16,000	1,000	S.D.	8,000	P.
Phenothioxin	80	32,000 (24)	(1,000)	U.	1,000	D.
Phenothiazine	3,300	32,000 (48)	(1,000)	U.	(1,000)	U.
10-Acetylphenothiazine	U.	U.	(1,000)	U.	(1,000)	U.
Phenothiazone	2,000	32,000 (72)	10,000	P.	8,000 16,000	L, P.
Thionol	U.	U.	1,000	U.	1,000	P.
Phenothiazine/						

Table I (continued).

(a).	(b).	(c).	(d).	(e).	(f).	(g).
Phenothiazine sulphoxide	250	U.	1,000	S.D.	4,000	P.
Lauth's Violet	27	U.	(1,000)	U.	1,000	D.
Methylene blue	40	8,000	(1,000)	U.	2,000 3,000	L, P.
Arecoline hydrobromide	1,700	2,000	1,000	P.	10,000,000	P.

Table II /

Table II.

Effect of some known anthelmintics, which are not included in Table I, on the free-living stages of Sclerostomes, A. lumbricoides and liver fluke in vitro; the same method and letters are used to show the effects as were used in Table I.

Compound.	Sclerostome larvae; concn. in faeces to kill 90%.	A. lumbricoides;		Liver fluke;	
		concentration,	effect.	concentration,	effect.
Nicotine sulphate 40%.	350	-	-	-	-
Nicotine	-	2,000 to 1,000	P.	200,000	P.
Carbon tetrachloride	80	2,000 to 1,000	P.	1,000	L.
Oil of Chenopodium	1,000	5,000	P.	5,000 20,000 to 10,000	L. P.
Copper sulphate	23	-	-	-	-
"Iodine vermicide"	1,250	-	-	-	-
Turpentine	180	-	-	-	-
Powdered areca nut	U.	-	-	-	-

Discussion. /

Discussion.

The results emphasise the need for screening compounds against a variety of helminths rather than against only one, as the tables show that the potency of many compounds varies considerably against the different helminths. In tests against Sclerostome larvae a few compounds are more effective against some genera than against others. Similarly, it is, of course, well known that *in vivo* some anthelmintics are very effective against some nematodes, but have little effect on others, and, perhaps, no effect on other helminths.

It appears, however, from Table I that if a compound has no effect on Sclerostomes, at 1:100 concentration, or on liver fluke, at 1:1,000 concentration, it has, in general, little or no effect against the other helminths *in vitro*, at 1:1,000 concentration. If this could be established on a larger scale, much work might be saved in future preliminary screening trials. The tables also suggest that, if the known anthelmintics had been subjected to *in vitro* testing before they were used as anthelmintics, they would not have been rejected during the preliminary screening, with the possible exception of areca nut, which is only used against tapeworms, and was only tested against Sclerostomes.

Sclerostome larvae, although pre-parasitic stages are used, may be particularly valuable for screening, because the greatest want in agricultural helminthology is for an anthelmintic, which will be effective against the immature parasitic stages of bursate nematodes, as well as against the adults, and which will have little, if any, effect on the host. It would be an additional advantage if the anthelmintic were suitable for incorporating in mineral licks or mixtures or in feeding stuffs, so that daily doses could reduce the damage to the host by the immature stages, which at present is usually unpreventable. However, tests to find suitable anthelmintics should, perhaps, use infective or exsheathed infective larvae, rather than all free-living stages.

Table I suggests that the aliphatic compounds containing iodine or bromine may be particularly effective against Sclerostome larvae. On the other hand, the aromatic halogen compounds are much less potent, except against liver fluke. The volatility of methyl iodide may account for the low values with A. lumbricoides and liver fluke, since they were tested in open containers, while the compound acted upon the Sclerostome larvae and vinegar eelworms in covered vessels.

Allyl/

Allyl iodide and isothiocyanate are very effective against all four helminths, and are more potent than allyl bromide or chloride. It is noteworthy that diphenylamine, 4-n-hexylresorcinol and gentian violet have so little effect against Sclerostomes compared with the other three helminths.

The lack of activity of the pyridines and quinoline towards vinegar eelworms, compared with their effect on the other nematodes, is interesting.

A comparison of the benzo-1:4-thiazine derivatives indicates that they are all paralysant, but not lethal, against liver fluke; however, 2:3-dihydro-3-ketobenzo-1:4-thiazine and its 6-chloro-derivative were very lethal against vinegar eelworms, whereas only the former was effective against Sclerostomes.

Only the Sclerostome and vinegar eelworm techniques show phenothiazine to be lethal, whilst phenothiazone is shown to be effective by all four techniques. Few of the other related compounds were outstanding in their in vitro anthelmintic potency.

Summary.

1. The results of four in vitro anthelmintic screening techniques are compared.

2./

2. Only thirteen out of the fifty-two compounds showed distinct anthelmintic activity in all four *in vitro* techniques. Thirty-four of the compounds were effective against free-living Sclerostomes at concentrations of 1:100 or less; twenty-eight were lethal to vinegar eelworms; twenty-four caused paralysis in *A. lumbricoides*, and thirty-five were lethal or paralysant to liver fluke.

3. Some results obtained by known anthelmintics with these *in vitro* techniques are included.

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